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*PHOSPHORYLATIVE AND NONPHOSPHORYLATIVE PATHWAYS OF ELECTRON TRANSFER IN RAT LIVER MITOCHONDRIA**

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Communicated by B. H. Willier, June 15, 1956

Separate pathways for the oxidation of reduced diphosphopyridine nucleotide (DPNH) and reduced triphosphopyridine nucleotide (TPNH) are known to exist in animal tissues. Although phosphorylation has been shown to be associated with oxidation of DPNH, the results with TPNH have been variously interpreted.[1] Hunter and Ford[2] have described rat liver mitochondrial preparations in which the oxidative and phosphorylative systems could be reversibly inactivated. Almost complete restoration of oxidative phosphorylation occurred upon the addition of diphosphopyridine nucleotide (DPN) and an appropriate substrate. We have found that such mitochondrial preparations do not contain either the oxidized or the reduced forms of DPN, but may contain traces of either TPNH or its oxidized form, TPN. It has been possible to study the single oxidative step from isocitrate to alpha ketoglutarate in these pyridine nucleotide–"depleted" mitochondria, by the addition of TPN. Using this approach, the question of phosphorylation associated with the oxidation of intramitochondrially generated TPNH has been investigated. In the course of this study, information has also been obtained which may be of significance in elucidating the function of TPNH and DPNH systems in regulating cellular respiration.

MATERIAL AND METHODS

Mitochondrial Preparation.—Rat liver mitochondria are ground gently with 6 volumes of 0.25 *M* sucrose + 0.02 *M* tris(hydroxymethyl)aminomethane (pH 7.5) in a loose-fitting glass homogenizer. The homogenate is centrifuged three times at 600 × *g*, for periods of 5 minutes each, to remove easily sedimentable materials. The homogenate supernatant is then centrifuged at 8,000 × *g* for 20 minutes and the mitochondrial pellet washed twice with 0.25 *M* sucrose + 0.02 *M* tris(hydroxymethyl)aminomethane (pH 7.5). Final suspension in the same medium was made so that 1 ml. represents 0.6 gm. of liver.

Depletion of Mitochondria.—To 2.4 ml. of the above mitochondrial suspension is added 0.6 ml. of 0.25 *M* sucrose + 0.1 *M* potassium phosphate (pH 7.5). The mixtures are incubated by shaking in a tube for 15 minutes at 30° and then returned immediately to ice, prior to use in the Warburg experiments.

Warburg Flask Additions.—The medium in the Warburg flasks contains the following substances in amounts to give the indicated final concentration in the 3.0-ml. total mixture: 0.0017 *M* ATP, 0.02 *M* NaF, 0.005 *M* $MgCl_2$, 0.01 *M* KCl, 0.005 *M* glycylglycine (pH 7.4), 0.001 *M* Na versenate (pH 7.5), potassium phosphate (pH 7.4), and 0.007 per cent cytochrome c (Mann Research Laboratories). The reaction mixtures are kept cold, and the glucose, 0.02 *M* final concentration, and 360 Cori units of yeast hexokinase are tipped in just as the flasks are placed in the 30° bath. Other additions are indicated in the tables. After equilibration for 10 minutes, the vents are closed. Oxygen uptake during the equilibration period is calculated from the 7-minute period after closing the vents, in a manner described by Hunter and Ford.[2] Oxygen uptake is measured for 30–40 minutes, and upon removal of the mixtures from the manometer, 1.5 ml. of 18 per cent trichloroacetic acid is added as quickly as possible. Phosphate and alpha ketoglutarate are determined in the trichloroacetic acid extract. The alpha ketoglutarate is determined by the method of Friedemann.[3]

RESULTS

Comparison of Phosphorylation Associated with the Oxidation of Mitochondrial-generated TPNH and DPNH.—Table 1 summarizes a typical experiment, comparing

TABLE 1

OXIDATIVE PHOSPHORYLATION IN UNDEPLETED AND DEPLETED MITOCHONDRIA

DPN and TPN additions: 1.5 μ moles per vessel
Isocitrate and glutamate additions: 30 μ moles per vessel
Mitochondria addition: 1.7 ml. of final suspension per vessel

Additions	Oxygen Uptake (μ atoms)	Phosphorus Uptake (μ moles)	P:O Ratio
Undepleted* mitochondria+glutamate	3.52	7.62	2.2
Undepleted mitochondria+isocitrate	9.95	29.9	3
Depleted† mitochondria+glutamate	0	0	0
Depleted mitochondria+isocitrate	1.1	0	0
Depleted mitochondria+DPN+glutamate	5.3	15.0	2.8
Depleted mitochondria+TPN+isocitrate	3.05	0.45	0.14

* DPN content of undepleted mitochondria per Warburg flask is 20 μg.
† DPN content of depleted mitochondria per Warburg flask is less than 1 μg., mitochondria having been preincubated with phosphate to remove pyridine nucleotides.

the relative activity of TPN and DPN in promoting phosphorylation coupled to oxidation. As can be seen from the data, in the normal, "undepleted" mitochondria, both isocitrate and glutamate oxidation result in phosphorylation. The "depleted" mitochondria still possess the capacity to promote phosphate uptake when glutamate is oxidized. Isocitrate oxidation, however, does not result in phosphorylation. Alpha ketoglutarate accumulates almost quantitatively as a result of the oxidation of isocitrate in the "depleted" particles. In a number of experiments with the "depleted" mitochondria, the addition of TPN and isocitrate results in a P:O ratio of zero or close to zero. When extremely large amounts of TPN are used, a ratio as high as 0.4 has been obtained. This mitochondrial preparation can convert TPN and DPN at a slow rate, and the slight phosphorylation observed with the high levels of TPN may be the result of the formation of small amounts of DPN.

The addition of DPN, as well as TPN, and isocitrate to the "depleted" mitochondria resulted in phosphorylation (Table 2). This phosphorylation was associated with an increase in oxygen uptake and in alpha ketoglutarate accumulation. The fact that phosphorylation is restored on the addition of DPN rules out the possibility that TPN is an inhibitor of oxidative phosphorylation.

TABLE 2

RESTORATION OF PHOSPHORYLATION WITH ISOCITRATE OXIDATION BY DPN*

Additions	Oxygen Uptake (μ atoms)	Phosphorus Uptake (μ moles)	P:O Ratio	α-Keto-glutarate Formed (μ moles)
Depleted mitochondria + DPN + glutamate	1.4	11.4	2.7	0.2
Depleted mitochondria + TPN + isocitrate	3.2	0	0	2.4
Depleted mitochondria + TPN + DPN + isocitrate	9.6	22.2	2.3	4.3

* Conditions the same as for Table 1.

It is of interest to note that oxygen uptake is much greater with isocitrate than with glutamate in the untreated mitochondria (Table 1). In the "depleted" system the rate of glutamate oxidation is somewhat faster than isocitrate. However, as indicated in Table 2, the addition of DPN to the TPN-isocitrate system results in a rate of oxygen uptake which is considerably faster than with glutamate and DPN.

Pyridine Nucleotide Transhydrogenase Activity of Mitochondria.—The increase in respiration, resulting from the addition of DPN in the presence of isocitrate and TPN, suggested the possibility that pyridine nucleotide transhydrogenase might be involved in the stimulation. This enzyme, which has been found in animal tissues, catalyzes the following reversible reaction:[4]

$$\text{TPNH} + \text{DPN} \rightleftarrows \text{DPNH} + \text{TPN}.$$

Evidence for the role of the transhydrogenase in liver mitochondria is given in studies of cytochrome c reduction, as illustrated in Figure 1. The rate with DPN and glutamate was found to be quite rapid when compared with the rate with TPN and isocitrate. TPN addition to the DPN-glutamate system actually slowed the reaction (Fig. 1). Addition of both DPN and TPN to the isocitrate reaction mixture produced a marked stimulation of cytochrome c reduction. It is of interest that the rate of cytochrome c reduction in the presence of isocitrate and DPN is somewhat faster than with isocitrate and TPN. This may be due to traces of TPN in the depleted mitochondria (see below).

The data in Figure 1 indicate that there is a fast reduction of cytochrome c by generated DPNH and a slow reduction by TPNH generated by isocitrate oxidation. The effect of the addition of DPN to the isocitrate reaction mixtures suggests a transfer of electrons from TPNH to DPN via the transhydrogenase with formation of DPNH, which could then be more efficiently oxidized by the cytochrome c. The slower rate observed when TPN is added to the DPN-glutamate system can also be attributed to transhydrogenase activity, since there is more TPN than cytochrome c present in the reaction mixture in Figure 1.

Additional support for the above view has been obtained from studies on the reduction of the dye 2,6-dichlorophenolindophenol (Fig. 2). In contrast to cytochrome c reduction, the rate of dye reduction with isocitrate and TPN is faster

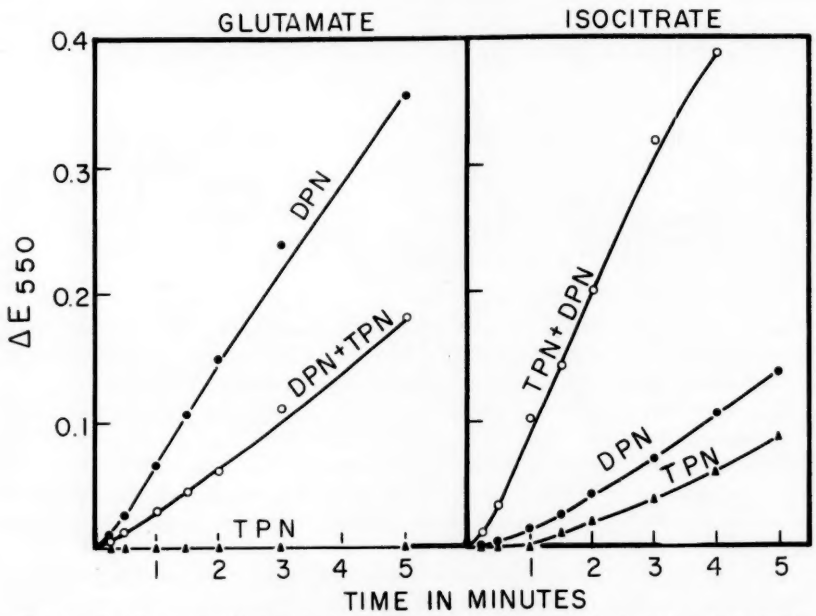

FIG. 1.—Pyridine nucleotide transhydrogenase activity of rat liver mitochondria as indicated by cytochrome c reduction. All reaction mixtures, 3.0 ml. total volumes, contained 2 mg. cytochrome c, 0.1 *M* phosphate buffer (pH 7.5), 0.001 *M* KCN, 0.003 *M* $MgCl_2$, 0.05 ml. "depleted" mitochondria, and 500 μg. DPN or TPN, except when both nucleotides were added: 1,500 μg. TPN with glutamate and 1,500 μg. DPN with isocitrate were used. The reactions were started with 5 μ moles of sodium glutamate or with 10 μ moles of *dl*-isocitrate.

than with glutamate and DPN. The addition of TPN to the DPN-glutamate reaction and of DPN to the TPN-isocitrate system has no effect on dye reduction. These experiments indicate that the TPN-isocitric dehydrogenase is a highly active system in mitochondria and that the limiting factor in the rate of isocitrate oxidation (in the absence of DPN) is the slow TPNH–cytochrome c system. More detailed experiments, directly showing the transhydrogenase activity, will be published elsewhere.

DPN Isocitric Dehydrogenase.—The stimulation of the oxidation of isocitrate by DPN might be the result of a DPN-specific isocitric dehydrogenase rather than the transhydrogenase. Indeed, the addition of DPN in the absence of added TPN to the "depleted" mitochondria occasionally promoted the oxidation of isocitrate. It is our belief, however, that the presence of residual TPN and the transhydrogenase are responsible for this oxygen uptake.

Plaut and Sung[5] report that rat liver mitochondria contain only traces, if any, of DPN isocitric dehydrogenase. In Figure 2 data are given which show that dye reduction with isocitrate and DPN is very slow. Furthermore, we have shown by direct test that the "depleted" mitochondria do not reduce DPN when isocitrate is the substrate; under the same conditions TPN is rapidly reduced.

These findings strongly indicate that DPN isocitric dehydrogenase is not involved in stimulation of oxygen uptake by DPN when isocitrate is the substrate.

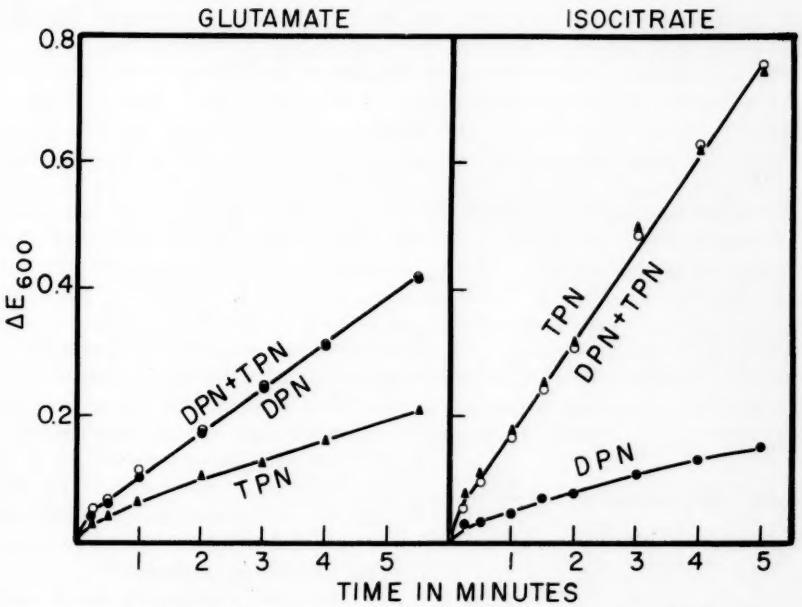

FIG. 2.—Dye reduction by depleted rat liver mitochondria. Reaction mixtures were the same as those described for Figure 1, with the omission of the cytochrome c and the substitution of 49 μg. 2,6-dichlorophenolindophenol.

DISCUSSION

From the results presented in this paper, it is evident that TPNH and DPNH oxidation proceed through separate pathways in rat liver mitochondria. DPNH oxidation through the cytochrome c system is a rapid one and leads to phosphorylation. On the other hand, the flow of electrons from TPNH to oxygen is a much more sluggish system, as indicated by the slow rate of cytochrome c reduction. The TPNH pathway does not appear to be associated with the generation of energy-rich phosphate bonds. It cannot be stated with certainty that there is a complete absence of phosphate uptake in the oxidation of TPNH, since P:O ratios of 0.1 to 0.4 have been observed with some mitochondrial preparations. Whether or not these ratios are significant remains for further study. Since a number of preparations have yielded no phosphate uptake, it is possible that in the case of TPNH oxidation no phosphorylation occurs even between cytochrome c and oxygen. The results we have obtained with TPNH may support the concept of Green *et al.*[6] and Reif and Potter[7] that separate electron-transferring systems may exist within the mitochondria.

Figure 3 presents a scheme which illustrates the role of pyridine nucleotide transhydrogenase in linking the TPN and DPN systems. Phosphorylation may be obtained with TPNH oxidation only after electron transfer to DPN via transhydrogenase. Our data suggest that the phosphate uptake associated with TPNH oxidation may occur through such a mechanism (see Table 2). The transhydrogenase, by shifting electrons from TPNH to DPN and thence to cytochrome c,

will stimulate the rate of oxygen uptake, since the DPNH–cytochrome c electron-transport system is much faster than the corresponding TPNH reaction.

Recently Jacobson,[8] of our laboratory, has shown that TPNH is the major pyridine nucleotide of carefully prepared rat liver mitochondria. Little DPNH is present in these mitochondria. The survival of TPNH during the isolation of mitochondria might be expected because of the relatively slow rate of oxidation of TPNH as compared with the oxidation of DPNH.

Langdon[9] has shown that TPNH can act as the reducing agent in the conversion of crotonyl CoA to butyryl CoA; DPNH cannot replace TPNH in this reaction, which leads to the synthesis of fatty acids (see Fig. 3). It is possible that the prime function of TPNH is to serve as a reducing agent. DPNH, on the other hand, is primarily oxidized and thereby gives rise to utilizable energy in the form of energy-rich phosphate bonds. The pyridine coenzymes may have different major functions. These different functions would then explain the required presence of two pyridine nucleotides rather than one in living systems. In this connection, it is of interest that Hoch and Lipmann[10] indicated the significance of the dual function of reducing systems arising from the oxidation of substrates for both the generation of energy-rich phosphate bonds and also for synthetic purposes as in fatty acid synthesis.

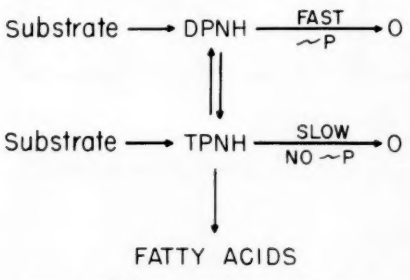

FIG. 3.—Pathways of electron transport.

The pyridine nucleotide transhydrogenase has previously been postulated to serve as a regulatory mechanism for the conservation of oxidative energy.[11] As illustrated in Figure 3, the shifting of electrons from DPNH to the TPN system would result in a lowering of phosphorylation; a shift in the direction of the DPN system would increase the level of phosphorylation. Work is now in progress in this laboratory on possible factors which may regulate the activity of the transhydrogenase system.

SUMMARY

By removing pyridine nucleotides from rat liver mitochondria and then adding TPN and isocitrate, it has been possible to show that TPNH oxidation does not result in phosphorylation.

The oxidation of DPNH (generated from the oxidation of glutamate) in the same preparation results in phosphorylation. Cytochrome c reduction by mitochondrially generated DPNH has been found to take place at a faster rate than with TPNH. The role of pyridine nucleotide transhydrogenase in rat liver mitochondria has been investigated, and a scheme that involves this enzyme as a regulator of respiration and of energy-releasing reactions is presented.

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CONDUCTANCE, VISCOSITY, AND VAPOR DENSITY FOR SEVERAL SALTS IN BROMINE AT 25°

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Communicated May 29, 1956

Equivalent conductances reported by Moessen and Kraus[1] for several salts in bromine, and viscosities reported by Rice and Kraus[2] for the same salts in the same solvent, show that, at high concentrations, salts are largely or even completely dissociated into their ions. It seemed worth while to study such solutions in somewhat greater detail. The chlorides and bromides form with one molecule of bromine rather stable complexes which melt at comparatively low temperatures. Compounds of the type $R_3NHX{\cdot}Br_2$ melt in the neighborhood of 35° and are readily handled at 25° in a supercooled condition. Here R represents an alkyl radical and X a halogen atom.

We have measured the conductance and the viscosity of solutions of $Am_3NHCl{\cdot}Br_2$ and $Me_3NHBr{\cdot}Br_2$ from comparatively low concentrations to the molten salts at 25°, and we have likewise determined the vapor densities over solutions of these two salts as well as of $Bu_4NBr{\cdot}Br_2$ and $Me_3NHCl{\cdot}Br_2$. Mercier and Kraus[3] have determined the phase relations for these systems.

Bromine was purified by the method described by Moessen and Kraus. The salts were prepared according to conventional methods. Bu_4NBr was recrystallized from ethyl acetate, m.p. 117°–117.5°. Conductances of this salt in water closely checked those of Martel[4] with another preparation. Me_3NHCl was recrystallized from ethanol-methanol mixtures, Me_3NHBr from acetone-methanol mixtures, and *i*-AmNHCl from acetone-ether mixtures.

Viscosities were measured by means of a modified Ostwald viscometer. The viscometer was provided with stopcocks, and the receiving bulb was provided with a 1-cm. tube which was capped. Salt could be introduced through this tube.

Concentrated solutions, as well as the supercooled fused salts, were made up in a special preparation tube from which they were transferred to the viscometer. These solutions were diluted by adding liquid bromine. Dilute solutions were made up by adding weighed salt samples to a known quantity of bromine in the viscometer. The bromine content of the viscometer was determined by weighing on a sensitive balance.

Four viscometers were constructed; they were calibrated against water and known sucrose solutions. Their efflux times are given in Table 1. The time

TABLE 1

EFFLUX TIMES OF VISCOMETERS

Viscometer Number	Liquid	Liquid Volume	Efflux Time (Sec.)
1	Water	11.87	895.0
2	40% sucrose	9.98	720.5
3	60% sucrose	12.60	988.7
4	60% sucrose	10.34	140.0

TABLE 2

DENSITIES OF SOLUTIONS OF SALTS IN BROMINE AT 25°

A. $Me_3NHBr \cdot Br_2$		B. $Am_3NHCl \cdot Br_2$	
Mole Fraction of Salt	Density (Gm/Ml)	Mole Fraction of Salt	Density (Gm/Ml)
0	3.102	0	3.102
0.05347	3.020	0.04128	2.717
.07194	2.991	0.05050	2.643
.1026	2.941	0.1151	2.283
.2018	2.778	0.1399	2.187
.2264	2.735	0.2909	1.777
.2621	2.680	0.3928	1.610
.3711	2.523	0.4452	1.555
.4336	2.446	0.5300	1.472
.6521	2.243	0.6390	1.398
.7882	2.149	0.7963	1.318
0.9214	2.075	0.8568	1.295
		0.9804	1.250
		1.006	1.244

of efflux was determined as a function of the volume of solution in the viscometers, so that it was not necessary to adjust the volume of the solution to a definite value. A plot of volume versus efflux time made it possible to obtain the efflux times of the standard solution for any degree of filling.

For Me_3NHBr, only viscometers 1 and 2 were required; for Am_3NHCl all four viscometers were employed. In going from one viscometer to the next, concentrations were adjusted to overlap, thus precluding the possibility that the two yielded different results.

The more concentrated solutions were made up by distilling bromine onto a known weight of salt in the viscometer. The melting points of highly concentrated solutions are above 25°, but, when melted, the solutions may readily be handled in a supercooled condition. After having determined the viscosity of the most concentrated solution, more bromine was added and the viscosity of the solution determined. This process was repeated until the limiting capacity of the viscometer was reached.

For the more dilute solutions, known weights of salt were added to a known

weight of bromine until a desired concentration was reached. Viscosity values are shown in the third column of Table 3 for rounded mole fractions of salt which is assumed to be complexed with one molecule of bromine.

TABLE 3

CONDUCTANCES, VISCOSITIES, AND VAPOR DENSITIES OF SOLUTIONS OF SALTS IN BROMINE

MOLE FRACTION	CONDUCTANCE	VISCOSITY	CONDUCTANCE-VISCOSITY PRODUCT	VAPOR DENSITY	REL. VAPOR-DENSITY LOWERING
		Me_2NHBr			
0.02	7.0	0.0125	0.0775	...	...
0.04	16.1	.014	0.22	1.900	0.008
0.06	23.2	.016	0.37	1.877	.020
0.08	29.6	...	...	1.835	.042
0.10	33.2	.0215	0.715	1.769	.076
0.14	36.8	...	...	1.490	.222
0.16	37.0	.0330	1.22	1.327	.307
0.18	36.3	...	...	1.166	.391
0.20	35.4	.0418	1.48	1.011	.472
0.30	29.9	.0639	1.91	0.474	.753
0.40	25.0	.0848	2.12	0.224	.883
0.50	21.1	.106	2.23	0.124	.935
0.60	17.7	.129	2.28	0.059	.969
0.70	14.6	.153	2.23	0.050	.974
0.80	11.8	.183	2.16	0.034	.982
0.90	9.45	.215	2.03	0.023	.988
1.0	7.45	0.254	1.89	0.017	0.991
		Am_3NHCl			
0.01	1.40	...	...	...	...
0.02	2.40	...	...	...	...
0.03	3.25	...	...	...	...
0.04	3.75	0.0093	0.035	...	...
0.05	4.04	...	...	1.909	0.003
0.07	4.26	...	...	...	...
0.08	4.26	0.0305	.130	...	...
0.10	3.95	0.0481	.190	1.735	.094
0.15	2.80	0.104	.290	1.406	.266
0.16	...	...	...	1.331	.305
0.20	1.73	0.205	.355	1.005	.475
0.25	1.05	0.386	.405	0.622	.675
0.30	0.680	0.654	.445	0.391	.796
0.35	...	...	.470	...	...
0.40	0.460	0.998	.459	0.184	.904
0.42	...	...	.458	...	...
0.45	...	...	.470	...	...
0.50	0.245	2.00	.490	0.090	.953
0.60	0.190	2.59	.492	0.052	.973
0.70	0.140	3.32	.465	0.031	.984
0.80	0.110	4.05	.445	0.019	.990
0.90	0.090	4.83	.435	0.015	.992
1.0	0.075	5.80	0.435	0.013	0.993

Densities of solutions were determined by means of a pycnometer. Values are shown in Table 2 for different mole fractions of salt which is assumed to contain one molecule of bromine. Vapor densities were determined by weighing a bulb of about 350-cc. capacity, filled with vapor which was in equilibrium with a known solution. These solutions were prepared in a cylindrical tube of about 100-cc. capacity which was provided with a stopcock and a small capped tube through which salt could be introduced. Bromine was distilled into this tube under vacuum conditions. After distillation, the tube was warmed and shaken until its contents became homogeneous.

The vapor-density bulb was then attached to the tube containing the solution and exhausted. Then the solution was cooled to dry-ice temperature, connection was made with the vapor-density bulb, and the whole apparatus was thoroughly exhausted in order to remove all traces of air. The solution was then brought to 25° and maintained there with occasional shaking until equilibrium was established. The vapor-density tube was then detached and weighed. As was shown by measurements of vapor densities at different pressures of bromine, the vapor density, at the low pressures of the present experiments, is a linear function of pressure. Accordingly, the relative vapor-density lowering $(d_0 - d)/d_0$ is equal to the vapor-pressure lowering $(p_0 - p)/p_0$. The density of saturated bromine vapor at 25° was found to be 1.915 gm/liter.

By weighing under hexane, the densities of Am_3NHCl and Me_3NHBr were found to be 0.933 and 1.570, respectively. For Bu_4NBr and Me_3NHCl we adopted the density values of Rice and Kraus, 1.225 and 1.099, respectively.

The conductance cell consisted of two cylindrical tubes of about 50-mm. diameter which were connected at the bottom with a 10-cm. length of tube about 7 mm. in diameter. The cylindrical tubes were provided at the top with ground-glass stoppers which carried the electrodes at the bottom of the two large tubes. The cell was provided with suitable stopcocks and could be attached to a source of bromine and the vacuum system. The cell had a constant of approximately 36, which was independent of the position of the electrodes or the volume of solution in the cell. Means were provided for stirring the solution. Concentrated solutions were made up by adding bromine to a known weight of salt; dilute solutions, by adding salt to a known weight of bromine. The cell contents were determined by weighing on a sensitive balance.

The results of our measurements of conductance, viscosity, and vapor-density lowering for Am_3NHCl and Me_3NHBr are shown in Table 3, where concentration is expressed as mole fraction of salt (containing one molecule of bromine) at rounded values as interpolated from large-scale plots. Values of equivalent conductances are given in the second column, of viscosity in the third column, and of the conductance-viscosity product, $\Lambda\eta$, in the fourth column. Vapor densities are given in the fifth column and relative vapor-pressure lowerings in the sixth column. Vapor densities and vapor-density lowerings for Bu_4NBr and Me_3NHCl are given in Table 4.

In Figure 1 values of Λ are shown plotted as a function of mole fraction, x, of salt assumed to be $Me_3NHBr{\cdot}Br_2$ and $Am_3NHCl{\cdot}Br_2$, respectively. Points shown on these, as on all other plots, are experimental, not interpolated.

The conductances of the bromide and the chloride pass through maxima of 37.0 and 4.26 at $x = 0.11$ and 0.075, respectively. For the fused salts at $x = 1$, the values of Λ are 7.45 and 0.075, respectively. It is of interest to note that the conductances of $Me_3NHCl{\cdot}Br_2$ and $Bu_4NBr{\cdot}Br_2$ pass through maxima of 15.75 and 13.9 at $x = 0.11$ and 0.08, respectively.[1] These salts are all rather strong electrolytes in bromine solution. It will also be noted that while the conductance of Am_3NHCl is markedly lower than that of Me_3NHBr at the maximum, that of Bu_4NBr is almost as large as that of Me_3NHCl. The reason for this, as we will see, is due to the much lower conductance of the chloride with respect to that of the bromide ion.

The viscosities of solutions of Me_3NHBr and Am_3NHCl are shown graphically

in Figure 2. Both plots show a slight inflection in the concentrated regions. The viscosities of the fused $Me_3NHBr{\cdot}Br_2$ and $Am_3NHCl{\cdot}Br_2$ ($x = 1$) are, respectively, 27 and 620 times that of bromine (9.435×10^{-3} poise). The high viscosity of the chloride is due to the presence of the large tri-*iso*amylammonium ion. At a concentration of 1.0 molar, the relative viscosities of solutions of Me_3NHCl, Me_3NHBr, Am_3NHCl, and Bu_4NBr are, respectively, 1.6, 1.7, 3.4, and 3.9.

TABLE 4

VAPOR DENSITIES OF SOLUTIONS OF SALTS IN BROMINE

A. Me_2NHCl			B. Bu_4NBr		
Mole Fraction	Vapor Density	Rel. Vapor-Density Lowering	Mole Fraction	Vapor Density	Rel. Vapor-Density Lowering
0.04	...	...	0.04	1.882	0.017
0.06	...	0.008	.06	1.819	.050
0.08	...	...	.08	1.727	.098
0.10	1.842	.038	.10	1.599	.165
0.14	1.676	.125	.14	1.254	.345
0.16	1.515	.209	.16	1.048	.453
0.18	1.316	.313	.18	0.837	.563
0.20	1.160	.394	0.20	0.674	0.648
0.30	0.651	.660			
0.40	0.393	.795			
0.50	0.236	.877			
0.60	0.149	.922			
0.70	0.100	.948			
0.80	0.071	.963			
0.90	0.057	.970			
1.0	0.048	0.975			

The conductance maximum and the rapid decrease of conductance at higher concentrations are due to the increasing viscosity of the solutions. At low concentrations, where the viscosity of the solutions differs little from that of the pure solvent, we are able to approximate the degree of ionization of the electrolyte by means of the equivalent conductance of the solution, for the values of Λ_0 may always be approximated by means of Walden's rule. At higher concentrations, where the viscosity of the solution becomes much greater than that of the pure solvent, this can no longer be done. However, the degree of ionization may again be approximated by means of Walden's rule. According to this rule, which is quite generally applicable, the conductance of the solution is proportional to the reciprocal of the viscosity. Thus the change in the product, $\Lambda\eta$, yields an approximation of the change in the degree of ionization. If ionization is complete, the conductance-viscosity product should remain substantially constant or vice versa. If the product remains constant on changing the condition of the system, the electrolyte may be considered to be completely dissociated into its ions. If the product decreases, the degree of ionization must be decreasing and vice versa.

In Figure 3 are shown plots of the $\Lambda\eta$ products for $Am_2NHCl{\cdot}Br_2$ and $Me_3NHBr{\cdot}Br_2$, as a function of mole fraction. For these salts as supercooled liquids, $\Lambda\eta$ has the values 0.470 and 1.89, respectively. For Bu_4NCl in ethylene chloride, $\Lambda_0\eta = 0.512$. The value of $\Lambda\eta$ for $Am_3NHCl{\cdot}Br_2$ in the liquid state is much the same as that for similar salts in ethylene chloride at infinite dilution. This accords with the assumption that in the fused state the electrolyte is completely dissociated into its ions.

The value of $\Lambda\eta$ for $Me_3NHBr{\cdot}Br_2$ is unexpectedly high; for Me_4NBr in ethylene

chloride, $\Lambda_0\eta = 0.600$. It seems that the bromide ion is a much better conductor than the chloride ion in these fused salts as well as in their solutions in bromine.

We do not have data on the conductance and viscosity of Me_3NHCl in the fused state, but from the data of Rice and Kraus[2] it appears that $\Lambda\eta$ approaches a value of about 1.0 in the limit. So, also, the conductance of Bu_4NBr at about 1 molar, the highest concentration measured, is practically as large as that of Me_3NHCl, although the viscosity of the bromide solution is several times greater than that of the chloride.

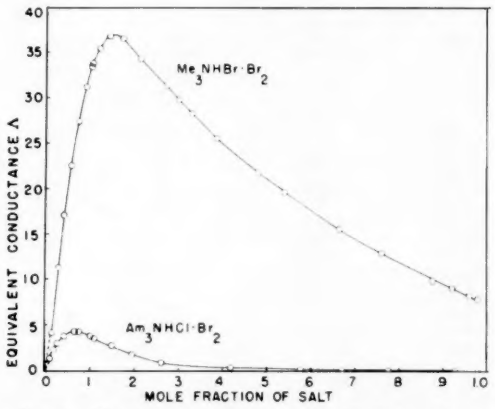

FIG. 1.—Equivalent conductances of salts in bromine.

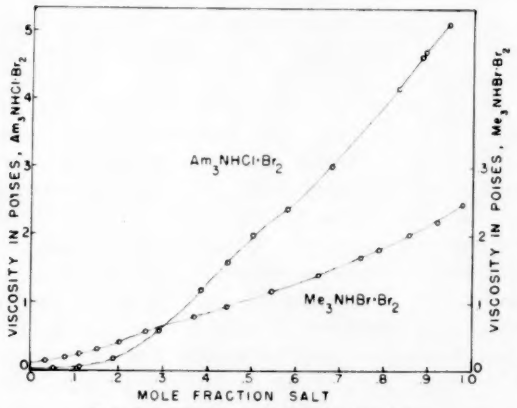

FIG. 2.—Viscosities of salts in bromine.

If Walden's rule holds even roughly, it follows that in the fused salts, as well as in their solutions in bromine, the complexed bromide ion has about four times the conductance of the corresponding chloride ion. In the fused salt the bromide ion consists of the bromine atom with a single charge for the whole. The ion may well have a linear structure. In any case, any one of the bromine atoms may carry the charge at any given instant. The ion thus behaves much like the hydrogen, or,

better, the hydroxyl, ion in water. On the other hand, in the chloride ion, $Cl{\cdot}Br_2$, the charge is definitely localized in the chlorine atom; there is no exchange of charge with the adjoining bromine atoms. Accordingly, the chloride ion has a normal conductance.

It has been shown on the basis of phase studies that bromide and chloride ions form a series of complexes with bromine molecules.[3] In some cases these com-

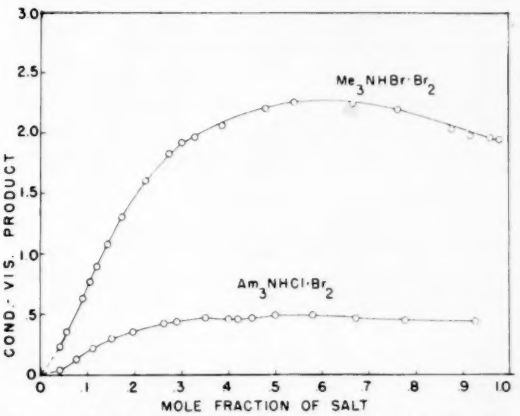

FIG. 3.—Conductance-viscosity products for salts in bromine.

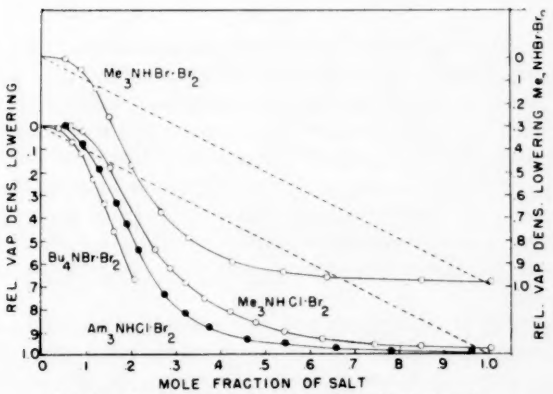

FIG. 4.—Vapor-density lowerings for salts in bromine.

plexed ions contain as many as four bromine molecules. As bromine is added to the fused salt, it goes, for the most part, to form complexes, and but little remains as free bromine in solution. This is clearly shown by the very low vapor pressure of the concentrated solution, as illustrated in Figure 4. Up to a mole fraction of about 0.4, practically all the bromine is combined with the halide ion. Thus we are not dealing with a single fused salt; rather, we are dealing with a mixture of salts of varying composition. This accounts for the fact that the $\Lambda\eta$ product does not remain constant or decrease on initial addition of bromine to the fused salt.

If we know the value of the conductance-viscosity product of the salt when completely ionized (we shall designate this as $(\Lambda\eta)_0$), we can approximate the degree of its association. The degree of ionization is given by the expression

$$\gamma = \Lambda\eta/(\Lambda\eta)_0$$

and the degree of association is $1 - \gamma$. The error in the evaluation of $1 - \gamma$ by this method will be large when $1 - \gamma$ is small. However, when $1 - \gamma$ is large, a fair approximation may be made.

We cannot determine just when ion association sets in. For the present calculation, we shall assume that it sets in at $x = 0.4$ for Me_3NHBr and at $x = 0.35$ for Am_3NHCl. The corresponding values of $(\Lambda\eta)_0$ are, respectively, 2.12 and 0.470.

In Table 5 are shown values of $1 - \gamma$ for the two salts computed on the basis of the method just outlined. Ion association sets in as soon as the concentration of

TABLE 5

DEGREE OF ASSOCIATION, $1 - \gamma$, OF SALTS IN BROMINE

A. Me_3NHBr $(\Lambda\eta)_0 = 2.12$ at $x = 0.4$		B. Am_3NHCl $(\Lambda\eta)_0 = 0.47$ at $x = 0.35$	
x	$1 - \gamma$	x	$1 - \gamma$
0.4	0.00	0.35	0.00
.3	.095	.30	.06
.2	.30	.25	.14
.16	.42	.20	.25
.10	.66	.15	.38
.06	.82	.10	.60
.04	.90	.08	.72
0.02	0.96	0.04	0.93

free bromine becomes significant. Trimethylammonium bromide complexes with a total of three molecules of bromine or two molecules above that of the fused salt, $R_3NHX \cdot Br_2$. At $x = 0.33$, there are two molecules of bromine above that of the fused salt. Since two of them may be complexed to the bromide ion, it seems probable that only about one molecule of bromine is present in solution in the free state. It is possible that complexes are formed containing more than three molecules of bromine. The degree of association at $x = 0.3$ is approximately 10 per cent. Seemingly, a very slight disturbance of the structure of the liquid electrolyte causes a marked interaction between individual ions with the consequent formation of ion pairs.

The high stability of the complex with one molecule of bromine is shown by the low vapor pressure of bromine over the fused salts. For a bromine content just below that corresponding to one molecule of bromine, the relative vapor densities lowerings for Me_3NHCl and Me_3NHBr were found to be 0.975 and 0.991, respectively. For Am_3NHCl at $x = 0.965$, the relative vapor density lowering was found to be 0.993. The last-named compound is the most stable of the three.

The marked stability of the higher complexes is illustrated by the relative vapor-density lowerings, as shown in Figure 4. The vapor pressure falls off most sharply for Bu_4NBr and least sharply for Me_3NHCl; Am_3NHCl is next to the tetrabutyl compound; evidently the nature of the substituted ammonium ion has an appreciable effect on the stability of the complex with bromine.

The broken lines in Figure 4 correspond to the vapor-pressure changes if Raoult's

law held. They are drawn on the assumption that the salt component contains only one molecule of bromine. This, of course, is not true, for we know that stable compounds of higher order are formed. Nevertheless, a comparison of the observed vapor-pressure lowerings with those predicted by Raoult's law yields information concerning the state of the electrolyte in dilute solutions. In all cases the vapor-pressure lowering in dilute solutions is much below that required by Raoult's law. This indicates that the molecular weight in dilute solution is greater than the formula weight. In short, it indicates that in the more dilute solutions the dipoles interact to form quadripoles and perhaps more complex structures.

In the present investigation, conductance measurements for Me_3NHBr were carried down to a molar concentration of only 0.9. The data therefore do not enable us to follow the association process to lower concentrations. However, Moessen and Kraus[1] have measured the conductance of Me_3NHCl to 0.036 N, and Rice and Kraus[2] have determined viscosities over the same concentration range. The highest concentration to which measurements were carried was 3.0 N, which corresponds to $x = 0.33$. The value of $\Lambda\eta$ at this concentration was 0.976 and was leveling off rapidly. For this salt we shall assume a value of 1.1 for $(\Lambda\eta)_0$. This value may be somewhat high, but it is a sufficiently close approximation for our present purpose.

In Table 6 are given values of Λ, η, $\Lambda\eta$, and γ, for Me_3NHCl at the lowest concentration measured and the concentration of maximum conductance. This concentration corresponds to $x = 0.10$. In the last line are given values of the ratio of the various quantities. At a concentration of 2.01 molar ($x = 0.10$), the salt is

TABLE 6

COMPARISON OF γ VALUES FOR Me_3NHCl AT TWO CONCENTRATIONS

	C	Λ	$\eta \times 10^3$	$\Lambda\eta$	$100\ \gamma$
	0.0362	1.217×10^{-2}	0.950	1.16×10^{-4}	0.0106
	2.011	15.75	2.50	0.394	36
Ratio	55.5	1,216	2.62	3,300	3,300

dissociated into its ions to the extent of 36 per cent. At 0.0362 N, γ is only 0.0106 per cent. The degree of dissociation has decreased in the ratio of 3,300/1, while the concentration has decreased in the ratio of 55.5/1. Obviously the degree of association of the ions increases enormously as the concentration of the solution decreases.

There is an equilibrium between ions and ion pairs, and there is a relation between the concentrations of the reactants and that of the product. But the relation between these quantities is not that postulated by the law of mass action; it is just the reverse. It is, of course, the reverse of what occurs in dilute electrolyte solutions quite generally. There seems to be no escape from the conclusion that, in solutions of these electrolytes in bromine, the degree of ionization decreases with decreasing concentration, contrary to what we find in more dilute electrolyte solutions.

Without doubt, if the conductance measurements in bromine were carried to lower concentrations, a point would be reached where the conductance would pass through a minimum and thereafter increase in accordance with the law of mass action. All typical electrolyte solutions in solvents of lower dielectric constant

exhibit such behavior. It is of interest to note that potassium iodide in iodine at 140° has a minimum at 0.013 N and a maximum at 1.14 N.[1]

While data are lacking for more dilute solutions in bromine, we have extensive data for such solutions in benzene from 1 N to very low concentrations, where dissociation increases with decreasing concentration in accordance with the law of mass action. These data overlap those for the more dilute bromine solutions, particularly in the case of Me_3NHCl. What characterizes these solutions in benzene as in bromine is the enormous increase in ionization with increasing concentration.

The conductance, that is, the ionization, of tri-*iso*amylammonium picrate in benzene[5] closely parallels that of Me_3NHCl in bromine over the range for which we have data for salts in the latter solvent.

In Table 7 are given values of Λ for Me_3NHCl in bromine and Am_3NHPi in benzene at comparable concentrations. As may be seen from Table 7, the ionization

TABLE 7

COMPARISON OF Me_3NHCl IN BROMINE AND Am_3NHPi IN BENZENE

C	Λ	C RATIO	Λ RATIO
	A. Me_3NHCl in Bromine		
0.443 0.036	5.58 0.0122	12.3	457
	B. Am_3NHPi in Benzene		
0.35 0.03	0.0135 1.4×10^{-4}	11.7	900

of Am_3NHPi in benzene increases 900-fold for an 11.7-fold increase in concentration. This is approximately twice that of Me_3NHCl in bromine over much the same concentration interval. It follows that the phenomena occurring in benzene are much the same as those in bromine. We may therefore employ data available for benzene solutions to assist in interpreting phenomena occurring in bromine solutions. We have extensive data relating to electrolytes in benzene.

Tri-*iso*amylammonium picrate is a relatively weak electrolyte in benzene. Its dissociation constant[5] is 4×10^{-21}. Its conductance passes through a minimum[6] at $C = 1 \times 10^{-3}$ N with a conductance of 2.7×10^{-7}. Its conductance passes through a maximum[7] at 1.08 N at a value of 0.0475. At 0.01 N the picrate is associated to quadripoles to the extent of 6 per cent and to 58 per cent at 0.1 N.[8] In the concentration range 0.01–0.1 N, the conductance, and therefore the ionization, increases 329-fold. In this concentration range, the molecular species are ion dipoles with some quadripoles, and the ions are simple ions and triple ions and possibly some ions of higher order due to interaction of quadripoles with simple ions.

The conductance in the immediate neighborhood of the minimum may be accounted for on the basis of triple-ion formation, but the enormous increase in conductance between 0.01 and 0.1 N cannot be accounted for in this way. Moreover, complex ions can be formed only by interaction of simple ions with ion dipoles or quadripoles, and the large increase in conductance therefore requires a large increase in simple ions. It would seem that the mass-action equilibrium, which applies at lower concentrations, suffers a reversal in the neighborhood of the concentration of the conductance minimum, as a result of which the dissociation of ion

dipoles increases with increasing concentration. It needs to be pointed out that this does not apply to the association of dipoles to quadripoles.

It may prove instructive to consider the behavior of another type of electrolyte in benzene. Tetra*iso*amylammonium thiocyanate is a relatively strong electrolyte[7] in benzene ($K = 1.93 \times 10^{-17}$).[5] Its conductance passes through a minimum at 2×10^{-5} N, where $\Lambda = 1 \times 10^{-4}$. Beyond the minimum, the conductance increases in a series of steps.[7] It rises sharply, levels off, and then rises again. The plot of log Λ versus log C shows three rises and two plateaus. At 1.14 N, the conductance is 0.338. Without doubt, if the concentration could be carried somewhat higher, the conductance would pass through a maximum, as do other similar salts.[7]

As shown by measurements of molecular polarization,[9] the ion dipoles begin to form quadripoles at a concentration as low as 5×10^{-5} N. At 1×10^{-3} N the association number of the salt, n, the number of formular weights per mole, is already 4.[5] From here on, n increases regularly to a value of 26 at 0.123 molal. Thereafter, n decreases sharply to a value of 10.5 at 0.43 molal.

It is clear that triple ions cannot be present to an appreciable extent at concentrations above 0.001 N. Neither can ion dipoles be present to any considerable extent; otherwise, the values found for the molecular weight of the solute could not be so high. The question is: What is the nature of the ions that conduct the electric current? It seems that there could be only two answers to this question. First, conductance is due to simple ions in equilibrium with charged aggregates. Second, conductance is due to charged fragments of the larger neutral aggregates.

If the first of these hypotheses is true, the ion dipoles in equilibrium with the simple ions must be very highly dissociated. It is, of course, possible that only one kind of ion dissociates from the aggregates. In that case, we would have simple ions of one sign and complex ions of the other. This possibility is not excluded. It might serve to account for the stepwise conductance increase.

If the second hypothesis is true, the solutions must contain a great variety of what we may call "fragment ions." These ions would have to be in equilibrium with a great variety of neutral aggregates. One difficulty with this hypothesis is that it does not serve to account for the large conductance increases found in the case of such salts as Am_3NHPi.

We shall not attempt to answer the questions that we have raised. Rather, our purpose is to call attention to a problem which, up to now, has been altogether neglected.

There is one more case that we shall consider briefly. Seward[10] has measured the conductance and the viscosity of Bu_4NPi in butanol at 93° from lower concentrations to the fused salt.

He found that there is no minimum in the conductance; it decreases continuously from the lowest concentration to the fused salt. The conductance-viscosity product, however, shows a pronounced and rather sharp minimum at 0.08 N, and Λ has the same value for the fused salt as it has for the solution at infinite dilution. The evidence seems to be conclusive that, at concentrations above 0.08 N, the degree of dissociation of the electrolyte increases with increasing concentration. The ion-pair equilibrium suffers a reversal at a relatively low concentration. This much is

clear; the phenomenon is not complicated by the formation of triple ions, as in other cases that we have examined.

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*STUDIES OF TYROSINASE PRODUCTION BY A HETEROCARYON OF NEUROSPORA**

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The tyrosinase of *Neurospora crassa* exists in two forms that differ in their thermostability.[1] This difference is inherited in a simple Mendelian way, the stable form of the enzyme being determined by a gene, T^S, and the labile form by its allele, T^L. The locus is on the right arm of the mating-type chromosome, 18 map units (uncorrected) distal to *adenineless*-35203 but proximal to *albino*-15300. As far as is known at present, the only other property in which the two forms of the enzyme differ is in the activation energy of thermal inactivation, which appears to be higher for the thermolabile form.[2] Measurements of several functional properties, including Michaelis constants for two substrates and relative turnover numbers, have not shown any significant difference between the two enzymes.[2]

Since the T^S-T^L alleles are recognized by a qualitative rather than a quantitative effect on the enzyme, an opportunity was presented for investigating the question of whether the alleles act independently in heterocaryons or whether they interact so as to produce a hybrid form of the enzyme or so as to show dominance of one allele over the other. At the same time it was possible to carry out a test of the hypothesis that tyrosinase synthesis is mediated by self-duplicating cytoplasmic particles. The principle of this test will be explained in connection with the experimental results.

Strains and Methods.—The heterocaryon was produced by fusion of hyphae from T^S and T^L strains carrying, respectively, the mutant genes *adenineless*-35203 and *histidineless*-C140. The strains had previously been made nearly isogenic by six successive backcrosses of *ad*-T^S progeny from the cross *ad*-T^S × *hist*-T^L to the *hist*-

T^L parent. The nutritional markers served to stabilize the heterocaryon on minimal medium and to mark the nuclei in the second part of the experiment. Both homocaryotic components of the heterocaryon were of mating type A. The constitution of the heterocaryon was verified by showing that both original homocaryons could be recovered from a single hyphal tip.[3] Conditions of growth and of extraction and purification of the enzyme were as described previously.[1] Enzyme activities were determined colorimetrically with DL-DOPA as substrate.[1] Thermostability tests were carried out at 59° ± 0.02° C. and pH 6.

Tyrosinase Production by the Heterocaryon.—Measurements of the thermostability of tyrosinase produced by the heterocaryon and by the two parent homocaryons are shown in Figure 1. It is seen that thermal inactivation of the enzyme from the

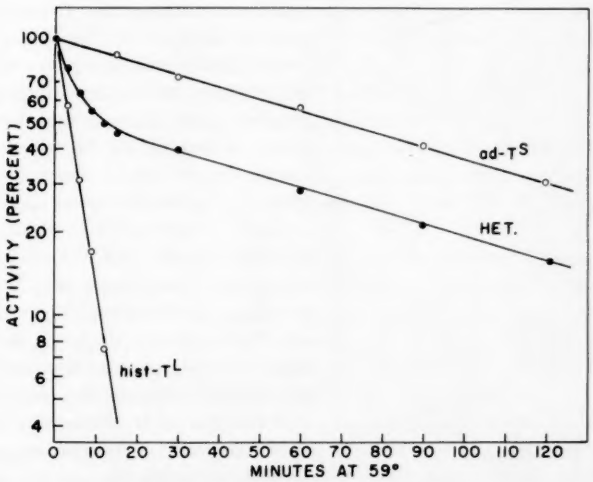

FIG. 1.—Thermal inactivation of tyrosinase from two homocaryotic strains and the heterocaryon between them. The half-life of the enzyme from *ad-T*^{S} is 70 minutes in this experiment; the half-life of the enzyme from *hist-T*^{L} is 3.4 minutes. In the case of the heterocaryon, the curve drawn through the experimental points is the theoretical for a mixture containing 52.3 per cent of tyrosinase of half-life 70 minutes and 47.7 per cent of tyrosinase of half-life 3.4 minutes.

homocaryotic strains is a first-order process, the half-lives of the two forms at 59° being 70 and 3.4 minutes, respectively, in agreement with previous determinations.[1] Inactivation of tyrosinase from the heterocaryon does not follow the first-order course expected in the case of dominance of one allele or of interaction to form an enzyme of intermediate stability. Rather, it closely follows the curve predicted on the basis of a mixture of the two original enzymes. The relative proportions of the two activities are deducible from the first-order tail of the curve, which represents the inactivation of the stable component of the mixture. By extrapolating this portion of the curve back to zero time, the initial amount of thermostable activity can be read off the y-axis; the amount of thermolabile activity and the expected course of the thermal inactivation are then easily derived. In this way it can be

shown that the curve of Figure 1 is that predicted from a mixture containing 52.3 per cent thermostable and 47.7 per cent thermolabile tyrosinase activity.

Resolution of the Heterocaryon.—To test the theory that production of the enzyme is mediated by self-duplicating cytoplasmic particles, the heterocaryon was resolved into its genetic components by cutting off and subculturing hyphal tips; a fraction of such tips can be expected to be homocaryotic by chance. Since there are no effective crosswalls in the hyphae of *Neurospora*, the genetically unlike nuclei of a heterocaryon share a common cytoplasm. On the hypothesis of self-perpetuating cytoplasmic agents, homocaryotic cultures derived in the manner described might therefore be expected to resemble the parent heterocaryon in their tyrosinase production, rather than the original homocaryotic strains.

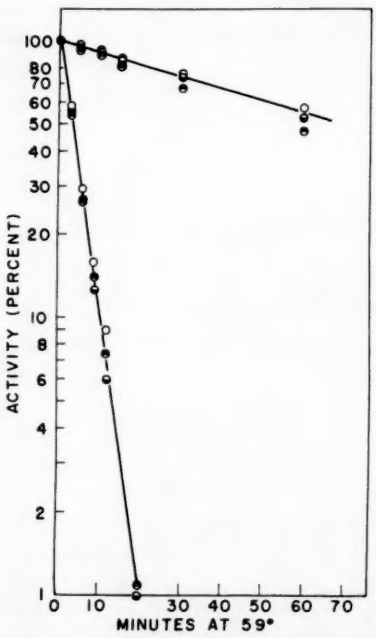

FIG. 2.—Thermal inactivation of tyrosinase from six homocaryons obtained by asexual resolution of an *ad-*T^S*/hist-*T^L heterocaryon. The curves are drawn to fit half-lives of 70 and 3.1 minutes, respectively.

Of 1,220 hyphal tips taken, 638 survived on transfer to minimal medium supplemented with adenine and histidine. Of these, 6 proved to be homocaryotic, as shown by the fact that they failed to grow on minimal medium. Transfer to medium supplemented with adenine or histidine showed that 3 of the isolates were adenineless (and therefore T^S) and 3 were histidineless (therefore T^L). These isolates were next grown in large cultures, and their tyrosinase was extracted, purified, and tested. The results, plotted in Figure 2, show that all the homocaryons produced only one form of tyrosinase, and this in accordance with the genetic constitution of the nucleus. The presence of 1 per cent of thermostable activity in the thermolabile preparations would have been detected, or the presence of 10 per cent of labile activity in the stable preparations. The half-lives determined for the six enzyme preparations were 3, 3.4, and 3.5 minutes for the enzyme from the histidineless cultures and 55, 69, and 74 minutes for the enzyme from the adenineless cultures. The stable form is generally more variable than the labile form in thermostability tests, owing to the fact that it is influenced to a greater extent by impurities in the preparations.[1] The effective impurity seems to be principally tyrosine, traces of which are apparently released by a proteolytic enzyme which contaminates some of the tyrosinase preparations. Like other tyrosinases, *Neurospora* tyrosinase is inactivated in reacting with its substrates, and the presence of tyrosine in the preparations causes an apparent decrease in the thermostability of the stable form; the labile form is hardly affected, since it is thermally inactivated too rapidly at 59° to engage in many catalytic cycles.

Discussion.—The absence of allelic interaction in the synthesis of tyrosinase by the heterocaryon parallels findings which have been made in the genetically similar case of sickle-cell anemia in man. It has been found that sickle-cell heterozygotes produce both the normal and the abnormal form of hemoglobin.[4] The same thing is true for the β_1- and β_2-lactoglobulins of cattle, which, on the basis of gene-frequency analysis of the data of Aschaffenburg and Drewry,[5] appear to be determined by a pair of allelic genes. It remains to be seen whether this will turn out to be a general rule for genes governing the structure of proteins, as would be expected on the template theory of gene action.

The failure to detect cytoplasmic transmission of tyrosinase-forming capacity in hyphal tip transfers is consistent with the known Mendelian inheritance of the thermostability difference. In order to account for the rarity of known cases of cytoplasmic inheritance compared with chromosomal heredity, it has been suggested that plasmagenes may be subject to decay in the germ line.[6] The present experiments lend no support to this idea, in so far as it draws a distinction between the germ line and the soma. Decay of the hypothetical plasmagenes in our case must be presumed to take place in asexual as well as sexual generations. It may be pointed out that the present experiments are not subject to the interpretation that persistence of the hypothetical plasmagenes depends on the presence of particular chromosomal genes (as in the case of kappa in *Paramecium*[7]), since both *T*-alleles support tyrosinase synthesis.

In view of the fact that the RNA (ribonucleic acid) of tobacco mosaic virus has recently been shown to possess genetic properties,[8] the continued failure to detect genetic activity in the cytoplasm—which contains RNA—in anything approaching the variety of activities associated with the chromosomes poses an interesting problem. One possible answer is that two structurally different forms of RNA exist, one capable of self-duplication and the other not.[9]

Summary.—A heterocaryon between two strains carrying, respectively, the alleles T^S and T^L, governing the thermostability of tyrosinase, was found to produce both forms of the enzyme. These alleles thus appear to act independently in the synthesis of the enzyme. The heterocaryon was resolved by means of hyphal tip transfers, without going through the sexual stage. No evidence was found for cytoplasmic transmission of tyrosinase-forming ability.

We wish to acknowledge the invaluable assistance of Mrs. Anne Kilim Nazarian. Dr. R. R. Jennings participated in the mapping of the *T*-locus. We are indebted to Dr. Dan Lewis, of the John Innes Horticultural Institution, for stimulating discussions.

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*THE GLUCOSE CONTENT OF THE DEOXYRIBONUCLEIC ACIDS OF CERTAIN BACTERIOPHAGES**

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The presence of glucose in the deoxyribonucleic acid (DNA) of the even-numbered bacteriophages of the T series was discovered by Jesaitis[1] and independently by Sinsheimer.[2] This glucose was shown to be associated with the hydroxymethylcytosine (HMC) in these nucleic acids as a glycoside substituent to the hydroxymethyl group (Sinsheimer;[2] Volkin[3]).

Further studies have indicated that the proportion of glucose is not the same in T2L and T4 bacteriophages and that the proportion of glucose seems to be connected with other recognizable traits of the bacteriophages (Streisinger and Weigle[4]). However, the mode of inheritance of these properties is unusual.

Methods.—All phage stocks were grown on *Escherichia coli* "B" according to the usual methods (Adams[5]) in the glycerol-casamino acid medium described by Fraser and Jerrel.[6] The stocks were purified by treatment with pancreatic deoxyribonuclease, followed by two cycles of differential centrifugation. DNA was obtained from the purified viruses by osmotic shock (Anderson *et al.*[7]), followed by high-speed centrifugation to remove "ghosts," deproteinization according to the method of Sevag *et al.*,[8] and alcoholic precipitation.

The molar proportion of glucose was determined by the ratio of the glucose content, as determined by the use of the anthrone reagent for glucose (Morris[9]), to the phosphorus content (Allen[10]). A correction for the color produced by the deoxyribose in the anthrone test was made by subtraction of the color produced by a calf thymus DNA solution (Gary and Klausmeier[11]) of the same phosphorus content. Recovery experiments with added glucose gave quantitative results.

Enzymatic Degradation of Bacteriophage DNA.—If DNA from any of these bacteriophage strains is degraded successively with pancreatic deoxyribonuclease and purified venom phosphodiesterase, about 65 per cent of the phosphorus of the digest can be recovered as mononucleotides.[2] The remainder is present as di-, tri-, and larger polynucleotides, all of which appear to contain HMC, as indicated by their ultraviolet absorption spectra.

If the DNA is that of a wild-type T2L, five mononucleotides are obtained: thymidylic acid, deoxyadenylic acid, deoxyguanylic acid, and two nucleotides of HMC, one with and one without a glucose substituent. The molar proportions of these mononucleotides are presented in Table 1, together with the proportion of each nucleotide in the T2L DNA which is obtained as a mononucleotide. It is clear

TABLE 1

MONONUCLEOTIDES FROM ENZYMATIC DIGEST OF T2L DNA

	Thymidylic	Adenylic	Guanylic	HMC	HMC-Glucose
Per cent of P of digest in mononucleotide	22.8	22.2	12.5	2.2	3.0
Per cent of nucleotide in digest recovered as mononucleotide	70	68	69	54	24

that the proportion of the glucose-substituted HMC mononucleotide found among the 65 per cent mononucleotide fraction is much lower than that of the other nucleotides, and thus, conversely, the enzyme-resistant polynucleotides must be enriched in this component. It seems likely that the presence of the glucose substituent, appropriately situated, confers resistance to phosphodiesterase action upon the polynucleotide.

The presence of the HMC nucleotide lacking glucose in such digests is clear evidence for incomplete glucose substitution of the HMC.

Analysis.—The nucleic acids of T2L and T4 bacteriophage and of the progeny of various crosses of these viruses have been analyzed for glucose content and have been enzymatically degraded to ascertain the possible presence of hydroxymethylcytosine lacking glucose substitution. The results are presented in Table 2.

TABLE 2

Virus Strain	Moles Glucose/ 100 Moles P	Presence of HMC Nucleotide, Not Glucose-substituted	Presence of Glucose-substituted HMC Nucleotide
T2L $r^+h^+u^+$	12.6	+	+
T4 r^+u	16.7	−	+
B×9 T2 $r^+h^+u^+$	17.1	−	+
B×9 T2 r^+h^+u	16.5	−	+
V-G T2 rhu^+	12.2	+	+
V-G T2 rh^+u^+	16.4	−	+

T4 differs from T2, among other traits, by the presence of a locus u conferring to its carrier a high resistance to inactivation by ultraviolet light (Luria;[12] Streisinger[13]). The strains B × 9 T2 are strains of T2 which, after one mating with T4, have been backcrossed to T2 nine times to make them as isogenic as possible with T2.[4, 13] It can be seen that they have acquired the "glucose property" of T4 and that it is not the u locus which is responsible for the glucose content. The V-G T2 stocks resulted from a single burst of a cross of $T2rh^+$ with B × 9 T2rh with delayed input of the second phage (Visconti and Garen[14]). These experiments are described in more detail in the next paper.[4] Under the conditions of delayed input, it can be seen that not all the progeny particles of the cross acquire the glucose property of T4.

Discussion.—In the wild-type T2L bacteriophage some (23 per cent) of the hydroxymethylcytosine is not substituted with glucose, while in T4 all the hydroxymethylcytosine is glucose-substituted. The inheritance of this "glucose property" is unusual in that it does not seem to be linked with any of the markers r, h, or u but does seem to be linked with the "bar" markers, which, in a cross T2 × T4 with simultaneous infection, are transmitted to the vast majority of the progeny.[4]

It is possible that the bipartite nature of the DNA of T2, suggested by Brown and Martin[15] and by Levinthal,[16] may be responsible for this unusual inheritance pattern.

Cohen (*Science*, **123,** 653–656 [1956]) has recently presented data suggesting a relationship between the glucose content of bacteriophage DNA and the *r* property. The data presented here do not support this relationship.

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*PROPERTIES OF BACTERIOPHAGES T2 AND T4 WITH UNUSUAL INHERITANCE**

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The first experiments on infecting bacteria mixedly with two different phages (Delbrück and Luria;[1] Delbrück[2]) were done with pairs of unrelated phages. They led to the discovery of exclusion, i.e., the finding that any one bacterial cell will yield mature particles of only one or the other of the parental types, never both. The nature of the exclusion mechanism is not understood.

Later it was found that exclusion is a function of the degree of relatedness of the two infecting phages. Phages which differ by only a few mutational steps are perfectly compatible (see Hershey[3]); not only do both phages multiply, but they also undergo extensive genetic recombination. Phages somewhat more distantly related show an intermediate behavior (Delbrück and Bailey[4]), the details of which have never been worked out.

The phages T2 and T4 belong to a very large group of related phages (Adams[5]). They are the phages whose genetic maps are better known (Hershey and Rotman;[6] Doermann and Hill[7]) than those of any other phage. They are also the pair for which genetic recombination and partial exclusion[4] were first observed. In the present paper we will describe the partial exclusion of T2 by T4 and will show that the "excludability" of T2 is itself a genetic trait of T2 which is completely excluded from the progeny. The excluding power of T4 is thus inherited in a novel way: it is imparted to the overwhelming majority of the progeny particles, irrespective of what other markers of either of the parents these particles inherit. Moreover, this excluding power of T4 is associated with two other characteristics. One of these is the characteristic that the particles carrying it, in contrast to those which do not carry it, plate with high efficiency on certain bacterial indicator strains.

The character is thus easy to score. The scoring of the other characteristic is laborious, but the character is of utmost theoretical interest: the particles with the excluding power of T4 have a different deoxyribonucleic acid (DNA) from those not carrying this power. In their DNA, 100 per cent of the hydroxymethylcytosine (HMC) is substituted with glucose, while, in the particles with the exclusion properties of T2, only 77 per cent of the HMC is substituted with glucose (Sinsheimer[8]).

MATERIALS AND METHODS

The phages used in these experiments were[9] T2 L and some of its mutant derivatives carrying the markers[10–12] *h* and r_{22}, and T4. The bacterial strains were *Escherichia coli* B and its mutant[12] B/2, as well as derivatives[13, 14] of *E. coli* K-12 called 112-12 and C-600. The general phage techniques are those described by Adams.[15]

EXPERIMENTAL

The bacteriophage properties having an unusual inheritance pattern are as follows:

1. *Efficiency of Plating (e.o.p.) on Strain 112-12.*—T4 when plated on 112-12 gives as many plaques as when plated on B, although the plaques on 112-12 are somewhat smaller. Plaques produced by *r* and r^+ phages on 112-12 are indistinguishable. T2 plated on young cultures of 112-12 gives only about 6 per cent as many plaques as on B; the plaques which do appear are so small as to be almost invisible. The T2 plaques which do appear on 112-12 contain phage identical to the parental T2 with respect to the low e.o.p. on 112-12. Strain C-600 exhibits similar relations to T2 and T4. Plaques formed on B by T4, upon replication with velvet (Lederberg and Lederberg;[16] Baylor[17]) on plates seeded with 112-12 and preincubated for an hour or on plates seeded with C-600 and preincubated for three hours, give plaques, while T2 plaques replicated under the same conditions give no plaques when replicated on either strain 112-12 or strain C-600.

2. *The Unequal-Yield Effect.*—In a cross of T2 × T4 on B bacteria with equal multiplicities, the markers introduced by T4 occur among the progeny particles with a frequency three to ten times higher than those of T2 (Streisinger[11]).

3. *The Glucose Content of DNA.*—In the DNA of T4, 100 per cent of the base 5-hydroxymethylcytosine (HMC) is glucose-substituted, while in the DNA of T2 only 77 per cent of the HMC is glucose-substituted. The studies on the definition of DNA are reported by Sinsheimer in an accompanying paper.[8]

In a cross between T2 and T4 most, if not all, of the progeny acquires the efficiency of plating of T4 (Table 1). $\overline{T2}$ (T2 bar) designates T2 phages which have acquired the e.o.p. of T4 through a cross with T4 or with another $\overline{T2}$. It is shown below that the phages which have acquired through a cross with simultaneous infection the e.o.p. of T4 on strain 112-12 have also acquired the other two properties. Collectively the three properties will be referred to as the "bar properties."

In the experiments to be described on the transmission of the bar properties, we utilized as donor of these properties, instead of T4, a $\overline{T2}$ strain which had been made more or less isogenic with T2. Such a strain had previously[11] been obtained, for the study of the *u* locus, by proceeding broadly as follows: First, $\overline{T2}$ was made

isogenic with T2 for most loci, except the *u* locus of T4 (controlling the level of ultraviolet sensitivity) through a series of eight backcrosses.[18] The *u* locus was replaced by the u^+ allele (from T2) in the last backcross. The progeny of the last (ninth) backcross will be symbolized as Bx 9 $\overline{T2}$.

TABLE 1
SINGLE BURSTS OF A CROSS OF T2 × T4*

Burst No.	No. T4 Plaques	No. T2 Plaques	No. Plaques Not Replicating on C-600	Burst No.	No. T4 Plaques	No. T2 Plaques	No. Plaques Not Replicating on C-600
1	1	30	0	6	24	7	0
2	11	38	0	7	106	133	0
3	36	28	0	8	18	32	0
4	42	51	0	9	65	55	0
5	26	11	0	10	43	59	0

* The cross was performed on bacteria of strain B grown to 10^8/ml, centrifuged, resuspended in buffer, and aerated for 90 minutes (to assure simultaneous infection; see S. Benzer, *J. Bacteriol.*, **63**, 59–72, 1952). The infected bacteria were distributed into individual tubes, so that the mean number of infected B per tube was 0.26. The contents of the individual tubes were plated on B + B/2 after incubation for several hours at 37° C. On these plates T2 particles form turbid plaques and T4 particles form clear plaques.

In a cross of T2 × Bx 9 $\overline{T2}$ the inheritance of the bar properties could thus be studied within an almost identical genetic background. The properties of T2, Bx 9 $\overline{T2}$, and T4 are contrasted in Table 2. It can be seen that the three bar properties introduced only once in Bx 9 $\overline{T2}$, nine crosses earlier, had persisted and had remained together. The properties studied are not closely linked to the *u*, *h*, or *r* loci, since, no matter what marker is carried by the progeny of the last backcross, the phages are identical with respect to the three properties (Table 2).

TABLE 2
EFFICIENCY OF PLATING, UNEQUAL-YIELD EFFECT, AND PER CENT OF GLUCOSE SUBSTITUTION COMPARED IN T2, Bx 9 $\overline{T2}$, AND T4 STRAINS*

Strain	Efficiency of Plating on Strain 112-12 (Per Cent)	Ratio of Test Marker to T2 Marker in Crosses with Approximately Equal Input	Per Cent HMC Substituted with Glucose[8]
T2$r^+h^+u^+$	12	1	77
Bx 9 $\overline{T2}r^+h^+u^+$	100	6† 3.6†	100
Bx 9 $\overline{T2}r^+h^+u$	100	6†	100
Bx 9 $\overline{T2}rhu^+$	100	10.6†	Not tested
T4r^+u	100	3.0†	100

* The efficiency of plating indicates the ratio of the number of plaques on strain 112-12 to the number of plaques on strain B. The plaques of T2 on strain 112-12 are barely visible.

The ratio of the number of markers of the strain studied to the number of markers of T2, among the progeny of a cross between the strain tested and T2, defines the unequal-yield effect. For instance, for Bx 9 T2$r^+h^+u^+$ the cross (Bx 9 $\overline{T2}r^+h^+u^+$) × (T2rhu) was performed with equal input multiplicity, and the ratio of h^+ to *h* in the yield measured.

† The multiplicity of $\overline{T2}$ to T2 in these experiments was equal, but the total multiplicity varied. It was noted in later experiments that the total multiplicity in such crosses had an effect on the ratio of T2 markers to T2 markers in the yield. Some of the variability in this series of numbers may be due to this factor.

In the crosses described thus far, the three bar properties seem to be imparted to every particle in the progeny. It seemed important to find out whether the bar properties were transmitted together under conditions in which not every progeny particle is converted into the bar form. These conditions can be realized by giving the non-bar parent a head start of several minutes. By delaying the input of the $\overline{T2}$ parent until 8 minutes after the initiation of development of T2 (Visconti and

Garen[19]), single bursts are obtained which contain a large number of non-bar T2 particles and very few T2 particles which are like T4 with respect to e.o.p. on strain 112-12. A typical burst of a cross T2$rh^+ \times \overline{T2}rh$ is described in Table 3. Two of its particles were analyzed, one h^+ replicating on 112-12 and one h not replicating on 112-12. The first one which was thus bar with respect to e.o.p. proved to be bar for glucose (Sinsheimer[8]) and for the exclusion properties. The other which was non-bar for e.o.p. was non-bar for glucose, non-bar for excludability, but was bar for excluding ability.

TABLE 3

YIELD OF A SINGLE BACTERIUM INFECTED WITH T2rh^+ AND SECONDARILY WITH $\overline{T2}rh$, YIELDING BOTH T2 AND $\overline{T2}$ PARTICLES*

No. h^+ Particles	No. h Particles	No. T2h^+ Replicating on 112-12	No. T2h Replicating on 112-12
136	3	1	0

* The cross was performed on bacteria of strain B grown to a concentration of 2×10^7/ml, centrifuged, resuspended in buffer at a concentration of 5×10^8, and aerated for 90 minutes. The bacteria were infected with an average multiplicity of two T2rh^+ particles per bacterium, and, after 4 minutes for adsorption, were diluted by a factor of 2 into concentrated nutrient broth and aerated at 37° C. After 8 minutes of development, $\overline{T2}rh$ particles were added at an average multiplicity of 400 per bacterium. Serum was added after 4 minutes to inactivate unadsorbed phage, and, after 2 minutes for serum action, the infected cells were distributed into individual tubes. After incubation for several hours at 37° C. to permit lysis to occur, the contents of the individual tubes were plated on B + B/2 to score the number of h particles. The number of $\overline{T2}$ particles was scored by replication onto plates seeded with strain 112-12 and preincubated for 1 hour. There were 0.15 bursts per plate.

The total exclusion of the non-bar properties and the partial exclusion of other markers from the progeny of a T2 $\times$ $\overline{T2}$ cross with simultaneous infection may be related. In order to understand how these total and partial exclusions take place and to find out what connection exists between them, an experiment on the unequal yield was performed and is described in Table 4.

TABLE 4

THE UNEQUAL-YIELD EFFECT IN A CROSS OF T2rh $\times$ Bx 9 $\overline{T2}r^+h^+$*

MULTIPLICITY OF T2rh	MULTIPLICITY OF Bx 9 $\overline{T2}r^+h^+$	Ratio of h/h^+ in Input	Fraction of Infective Centers Yielding No h	Effective Multiplicity of h	RATIO IN YIELD OF h/h^+	RATIO IN YIELD OF r/r^+
3.4	4.8	0.71	0.33	1.1	0.15	0.15

* The cross was performed on bacteria of strain B grown to a concentration of 10^8/ml, concentrated to 10^9/ml, and suspended in broth containing 10^{-3} M potassium cyanide (to assure simultaneous infection; see S. Benzer and F. Jacob, *Ann. Inst. Pasteur*, **84**, 186–204, 1953). After unadsorbed phage particles had been inactivated by antiserum, infective centers were plated before lysis on B + B/2. The rest of the culture was diluted and incubated for 2 hours at 37° C. The progeny thus obtained was plated on B + B/2.

The effective multiplicity m of the T2 parent was calculated from the fraction of infective centers, x, which liberated no particles with an h marker: $x = e^{-m}$.

The unequal yield of markers carried by the two parents of a cross of $\overline{T2}$ $\times$ T2 could be due to the complete elimination of a fraction of the T2 parental particles or to a relative decrease, by each particle, of its contribution of markers to the progeny (due to slower growth, for instance), or both effects might occur.

The effective multiplicity m of the T2 parent in a cross of T2rh $\times$ $\overline{T2}r^+h^+$ can be calculated from the fraction x of infected bacteria which yield progeny not having the h marker of the T2 parent, according to the Poisson equation: $x = e^{-m}$. It can be seen from Table 4 that a large fraction of the h markers of the T2 parent are completely excluded in mixed infections with $\overline{T2}$, since the effective multiplicity of the T2 parent is less than one-quarter of the input multiplicity. The ratios of h to

h^+ and r to r^+ markers in the progeny of this cross are identical (0.15), and similar to, though slightly less than, the ratio (0.23) of the effective T2 multiplicity (1.1) to the T2 multiplicity (4.8), indicating that the unequal yield in this cross is due principally to exclusion.

DISCUSSION

In simultaneous infections of B cells with T2 and T4 the markers of T2 are partly excluded; in addition, the excluding power of T4 is transmitted to most, if not all, of the progeny. Thus the "excludability" of T2 is itself completely excluded. Strains resulting from a cross of T4 × T2, and subsequently backcrossed to T2 nine times, so as to be virtually isogenic with T2, still retain the excluding power of T4.

Two other properties seem associated with the excluding power of T4: a high efficiency of plating on certain strains of bacteria as compared to a low efficiency of T2, and a difference in the DNA of the phages. The HMC of T4 is 100 per cent glucosylated, while that of T2 is 77 per cent glucosylated.

The backcrossed T4 derivatives which are more or less isogenic with T2 exhibit the first two properties of T4; the few strains tested contained 100 per cent glucosylated HMC as well. These properties are called "bar."

When infection of bar phage is delayed until several minutes after infection with non-bar phage, mixed bursts are obtained, containing bar and non-bar phage, in contrast to simultaneous infection. A particle which is bar with respect to one of the properties (e.o.p.) proved to be bar with respect to the other properties as well. A particle with the non-bar e.o.p. proved to be non-bar with respect to glucose, bar with respect to excluding ability, and non-bar with respect to excludability. These findings open the way to a study of the relations between the bar properties.

The partial exclusion of markers carried by the non-bar parent and the total exclusion of the non-bar property itself, in crosses with simultaneous infection, can be understood with the aid of a more or less conventional genetic model, as well as with other models. For instance, if the non-bar properties were determined by loci which are eliminated if—and only if—there is present with them in the bacterium a bar locus introduced by another phage, only particles carrying the bar loci would be observed among the progeny. Markers carried by non-bar particles could, however, be incorporated into vegetative particles with the bar loci by means of recombination and thus be "rescued" in a manner analogous to the rescue of markers of an ultraviolet inactivated particle in crosses with unirradiated phage. Loci close to the eliminated ones would, in this case, be expected to be rescued more rarely than distant loci. The study of the rescue of differently linked markers could yield information concerning this model. Such a model allows also an understanding of the separability of the bar properties.

It is tempting to try to relate exclusion of the non-bar properties to the different glucose content of non-bar and bar DNA. Cohen[20] has shown that DNA not containing HMC, and thus not containing glucose, is broken down more completely by certain enzymes than is DNA containing glucosylated HMC, and Sinsheimer[8] has shown that glucose-containing HMC is less readily separated from polynucleotides than non-glucosylated HMC. Thus non-bar DNA, containing less glucosylated HMC than bar DNA, could more readily be subject to a breakdown mechanism.

The gross chemical and biological differences found between T2 and T2 phage may be due to structures other than classical genetic loci. A structural differentiation of T2 DNA has been suggested by Levinthal,[21] who finds one large and several smaller pieces of T2 DNA, at least half of the larger piece being conserved throughout replication. A fractionation of the DNA of T2 into two fractions differing in their base ratio has been obtained by Brown and Martin[22] after a relatively gentle removal of DNA from the protein coat.

It is likely that a structurally bipartite T2 DNA is also functionally bipartite, consisting of material with the usual markers and of material with other functions. The difference in the glucose content of bar and non-bar DNA may be found to reside exclusively in the fraction of DNA not carrying markers. In that case the difference between bar and non-bar particles would be due not to loci but to a structurally separate piece of DNA. Construction of a detailed model involving such a separate piece at this time seems premature.

The material described here promises to be useful for an understanding of the mechanism of partial exclusion and thus for an understanding of the establishment of the metabolic regime leading to the production of the DNA of a particular phage, in competition with that of another phage and of the bacterium.

SUMMARY

1. Among the differences observed between the phages T2 and T4 are the following:

a) Markers of T2 are partly excluded in crosses of T2 × T4.

b) T4 plates with a high efficiency, while T2 plates with a low efficiency on certain strains of bacteria.

c) One hundred per cent of the 5-hydroxymethylcytosine (HMC) of T4 is glucose-substituted, while only 77 per cent of the HMC of T2 is glucose-substituted.

2. These three properties, unlike the usual genetic markers, are transmitted to a vast majority, if not all, of the progeny of crosses of T2 × T4. These properties are referred to collectively as the "bar" properties, and T2 particles having these properties are symbolized as $\overline{T2}$.

3. The progeny of a cross of T2 × T4, made subsequently more or less isogenic with T2 by repeated backcrosses, retain all three of the bar properties.

4. If progeny from single bacteria are infected with T2 and several minutes later are infected with $\overline{T2}$, the following observations are made:

a) The progeny contain particles with the T2 efficiency of plating as well as particles with the $\overline{T2}$ efficiency of plating.

b) Some of the particles seem to show a separation of the bar properties.

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*THE PRODUCTION OF TWO CHEMICALLY DIFFERENT TYPES OF CHROMOSOMAL BREAKS BY IONIZING RADIATIONS**

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The production of chromosome aberrations by ionizing radiations has been studied extensively, mainly because it is a particularly fruitful source of information on the quantitative relations between radiation dose and cellular damage. Primarily, radiation produces chromosomal breaks in direct proportion to the dose administered.[1] Ninety-five per cent of these breaks, as calculated by Lea[2] for *Tradescantia*, restitute, i.e., rejoin in their original configuration. The others may stay open as one-hit aberrations or rejoin with still other broken ends to form two-hit aberrations. These aberrations can be formed only if the two breaks are open simultaneously and in close proximity. If the radiation is administered at low intensity, thus allowing some of the breaks to restitute before others are produced, then fewer two-hit aberrations result than if the same dose were administered at higher intensity.[3]

Intensity studies of this nature in *Tradescantia* led Catcheside, Lea, and Thoday[4] to propose that two types of breaks are produced by the radiation: one group that

rejoins relatively rapidly and another that does so more slowly. Studies on the combined effects of near infrared and X-rays led Kaufmann and Wilson[5] to a similar proposal for *Drosophila* chromosomes.

Previous intensity experiments on the seed of *Vicia faba* indicated that X-ray-induced chromosome breaks stay open for at least two hours when the seed has been soaked in water.[6] However, breaks produced either in BAL[7] or *in vacuo*[8] remain open for only one-half hour. Wolff and Luippold[8,9] have postulated that since this type of break stays open for long periods of time and since respiration and ATP are necessary for its rejoining, it is the break of a strong, possibly covalent, bond. However, Mazia,[10] Steffensen,[11] and Levine[12] have reported that chromosomes are held together by the ionic bonds formed by the divalent cations of calcium and magnesium, and Steffensen[11] suggested that radiation breaks these bonds. Since breaks of ionic bonds cannot be expected to stay open for long periods of time, it is extremely unlikely that any of this type were observed in previous experiments with *Vicia*. The present experiments were therefore designed to ascertain whether or not another type of break existed which rejoined at a faster rate than did those previously described. Such short-lived breaks are indeed found, and their response to the chelating agent Versene suggests that they may be breaks of ionic bonds.

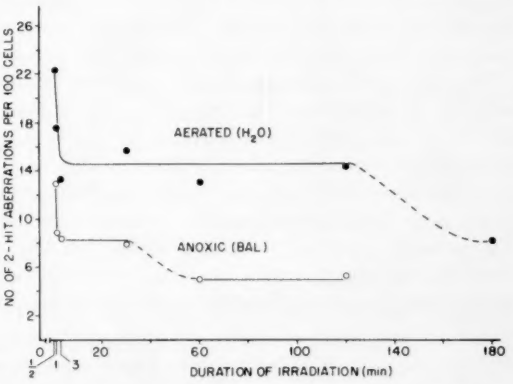

FIG. 1.—Two-hit aberrations produced by 600 r at different intensities.

Seeds of *V. faba* were soaked in distilled water for 24 hours and then peeled and arranged in a culture dish with their embryos facing upward. The dishes were exposed to 600 r of X-rays. For irradiation under anoxic conditions, the seeds were transferred to a 2×10^{-3} *M* solution of BAL one-half hour before exposure. In those final experiments that combined treatments with Versene and radiation, a different crop of seeds was used. Their response to radiation was the same as the crop used in the previous experiments if the seed coats were chipped and the seeds were soaked for only five hours. All irradiations were performed with a G.E. Maxitron tube operated at 250 kvp. with 3 mm. of Al filtration added (hvl., 0.4 mm. of Cu). The seeds were germinated as previously described[13] and fixed in C. E. Ford's modification of Flemming fixative (unpublished). Feulgen squashes were made, and at each point 300–500 metaphases of the first mitotic root-tip division were scored for rings and dicentrics.

The intensity curves in Figure 1 are characterized by plateaus that represent the intervals within which variation of the exposure time has no effect on aberration yield. These plateaus can exist only for times within which no appreciable rejoining occurs, for, otherwise, fewer open breaks would coexist at low intensities

than at high and the number of two-hit aberrations would be correspondingly altered. The length of the plateau wherein there is no diminution of two-hit aberrations is therefore a measure of the time the breaks stay open. This time, which is dose-dependent,[7] is at least two hours in seeds irradiated in water and one-half hour in those irradiated in BAL. If the duration of treatment exceeds these times, rejoining occurs and the typical intensity effect results. However, at radiation intensities higher than 200 r per minute, the number of aberrations produced in air (water) and under anoxia (BAL) increases above the plateau level. This indicates the presence of another type of break that rejoins (either restitutes or forms a two-hit aberration) very rapidly. If the radiation is administered at a very high intensity, many short-lived breaks are present in the cell simultaneously and are capable of yielding many two-hit aberrations. In seeds irradiated with the same dose, but at lower intensity, these breaks usually restitute, and only those that do not rejoin for a relatively long period of time remain. The latter are the breaks, described in previous reports from this laboratory, that are responsible for the plateau on the intensity curves, and are probably of a covalent nature. Thus two sharply distinct classes of breaks can be recognized, one with a lifetime of only about one minute and another that persists from one-half to two hours.

The very rapid rejoining of breaks observed only at the high intensities is consistent with the hypothesis that they are breaks of ionic bonds. For a further test of this hypothesis, seeds were soaked for varying times in a 0.001 *M* solution of ethylenediamine tetra-acetic acid (Versene). This concentration was chosen because it was effective in the studies of Mazia[10] on *Drosophila* and *Melanoplus* that led him to postulate that the chromosome consists of small units bound together by calcium and/or magnesium ions. Table I shows that low concentrations of Versene can break the chromosomes of *Vicia*, too. This can be interpreted as supporting evidence, in plant material, for Mazia's and Steffensen's models of the chromosome.

TABLE 1

PERCENTAGE OF ABERRATIONS PRODUCED BY SOAKING SEED IN VERSENE (0.001 *M*)

Total Cells	Time in Versene (Hours)	No. Aberrant Cells	Aberrations (Per Cent)
650	0	6	0.94 ± 0.38
750	3	27	3.60 ± 0.69
250	6	36	14.40 ± 2.4

We then reasoned that, since a three-hour soaking in Versene produces some chromosome breakage, this period must be long enough to insure penetration of the chelating agent into the cell. If subsequent irradiation at low intensities then produced "ionic" breaks in the chromosomes at the locations of calcium and/or magnesium ions, chelation would occur and the breaks, instead of restituting very rapidly, would stay open to become available for the production of aberrations. The results of a typical experiment of this type are reported in Table 2, which shows that pre-irradiation treatment with Versene in this instance gave a twofold increase in total aberration yield. It should be mentioned that the results of this type of experiment are unusually variable, both between slides and between experiments. Thus, although the increased aberration yield is qualitatively certain, the quantitative results may be poorly reproducible.

Steffensen's earlier observations[11] that the amount of chromosome breakage produced by combined calcium deficiency and radiation is more than the additive amount of both is consistent with these results. The same rationale used to interpret the Versene-radiation effect can also be used to interpret the calcium deficiency–radiation effect.

TABLE 2

PERCENTAGE ABERRATIONS PRODUCED BY COMBINED VERSENE (0.001 *M*) AND RADIATION TREATMENTS*

Slide No.	Treatment	Total Cells	Percentage Normal Cells	Percentage Fragments	Percentage Dicentrics and Rings
1	Versene, 3 hours	50	96.0	2.0	2.0
2		50	100.0	0.0	0.0
3		50	98.0	2.0	0.0
Total		150	98.0	1.33	0.67
1	600 r	50	66.0	24.0	16.0
2		50	80.0	12.0	10.0
3		50	80.0	8.0	12.0
4		50	78.0	12.0	10.0
5		44	72.7	9.1	13.6
6		56	71.5	16.6	17.85
Total		300	74.6	13.6	13.3
1	Versene 3 hours plus 600 r	50	44.0	44.0	32.0
2		50	46.0	44.0	32.0
3		50	58.0	28.0	34.0
4		50	64.0	22.0	16.0
5		50	64.0	22.0	18.0
6		50	82.0	8.0	12.0
Total		300	60.3	28.0	24.0

* Seeds soaked for a total of 5 hours.

It is possible, therefore, to interpret our results on the basis of two chemically different types of chromosome break produced by radiation. One type is a break of covalent bonds that require energy to close. The evidence accumulated here about the other type, although not conclusive, is consistent with the hypothesis that it is the break of an ionic bond.

It is interesting to note that the shapes of the *Vicia* intensity curves, after incorporation of the high-intensity points, are also reflected in the curves obtained when *Drosophila* sperm are irradiated. Haas *et al.*[14] have reported an increased number of translocations from irradiation at very high intensities. These translocations are analogous to the aberrations formed from "ionic" breaks in *Vicia*. These breaks disappear rapidly from the system by restitution, which is the reason they were never observed in previous experiments at relatively low intensities. There is no further intensity effect, because the other breaks, presumably of covalent bonds, stay open until the time of fertilization. These, then, are the equivalent of the breaks that give rise to the plateau of the *Vicia* curves. Since sperm respire very little, the breaks cannot rejoin until they are supplied with the requisite energy produced by respiration of the fertilized egg.

Thus in *Drosophila* sperm it may be postulated that the same two kinds of chromosome break exist and that the length of the plateau is determined not by the recovery of a "rejoining system" as in *Vicia*[9] but by the availability of the energy produced in the egg.

Summary.—Experiments on the seed of *V. faba* indicate that radiation-induced chromosome breaks may have two chemically different natures. One seems to be an ionic break, which closes very rapidly, as would be expected if only electrical factors were necessary for its rejoining. The other is covalent, and it stays open for long periods of time and needs a source of energy for the biosynthesis of the bonds formed in its closing. Such apparently unrelated experimental results as those obtained by the use of calcium deficiency,[11, 12] chelating agents,[10, 12] metabolic inhibitors,[8, 9] and high-intensity radiation[14] can be united by this concept of two types of chromosomal damage.

* Work performed under United States Atomic Energy Commission Contract No. W-7405-eng-26.

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*FREQUENCY OF SOMATIC MUTATION TO SELF COLOR IN MAIZE PLANTS HOMOZYGOUS AND HETEROZYGOUS FOR VARIEGATED PERICARP**

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Introduction.—Maize plants heterozygous for variegated pericarp and cob (P^{vv}) and a stable allele (e.g., P^{wr}, colorless pericarp and red cob) bear ears which are more heavily striped than those from homozygous variegateds. The basis of the difference is the number of mutations of P^{vv} to P^{rr} (self colored pericarp and cob) in pericarp tissue. Emerson[1, 2] who first established these facts, estimated that the single P^{vv} allele in $P^{vv}P^{wr}$ plants mutated to P^{rr} about 2.8 times as frequently as each of the P^{vv} alleles in variegated homozygotes. Tests which we and our colleagues have made with P^{vv} alleles of various geographic origins and on several genetic backgrounds have revealed that the numerical relationship differs in different backgrounds and, rarely, may even be reversed. Frequently, however, the difference is comparable to that which Emerson observed.

It will be shown in the present study that the difference in number of mutations

to self color in $P^{vv}P^{wr}$ and $P^{vv}P^{vv}$ plants can be accounted for satisfactorily in terms of dosage action of Modulator (Mp), a transposable element associated with the variegated allele.

The results of previous investigations[3] are consistent with the view that the variegated allele is a dual structure, comprising the P^{rr} gene and Modulator. It may be represented, therefore, by the extended formula $\overline{P^{rr}Mp}$. Mp, in this position, inhibits the pigment-producing action of P^{rr}. The mutation of variegated to self color consists in removal of Mp from the P locus, ordinarily by transposition to another site in the genome. It has been demonstrated also that one or more such transposed Modulators in a variegated pericarp plant markedly reduces, in an orderly way, the frequency of P^{vv} to P^{rr} mutations.[3, 4]

This latter fact suggests the reason why mutations to self color are less frequent per P^{vv} allele in P^{vv}/P^{vv} than in P^{vv}/P^{wr} plants. Each of the Modulators in the former genotype may be expected to reduce the frequency of P^{vv} to P^{rr} mutations in the homologous P^{vv} allele, whereas the single P^{vv} allele in heterozygotes is not subject to this modifying action.

If the mutation rate of a P^{vv} allele is equally influenced by an additional Mp in any position, including the locus of the homologous allele, then twice as many mutations to self color regularly will occur in homozygous variegateds as in heterozygous variegateds carrying a transposed Mp in hemizygous condition. This result would be expected because there are two P^{vv} alleles which can undergo mutations to P^{rr} in the former genotype as compared with only one in the latter, and one Modulator is present in each case, not counting the one at the mutating allele. The experimental data obtained in the present study verify this prediction.

Materials and Methods.—The maize strains used were variegated counterparts of a commercial yellow dent inbred line known as W23 (P^{wr}). The initial outcross to variegated pericarp had been followed by five backcrosses of the P^{vv}/P^{wr} segregates to W23, resulting in a high degree of uniformity in genetic background. Three genotypes then were selected from this inbred stock, differing in the expression of variegated pericarp as follows: (1) $\overline{P^{rr}Mp}/P^{wr}$, designated "medium variegated"; (2) $\overline{P^{rr}Mp}/P^{wr}$ + tr $Mp/-$, designated "light variegated"; (3) $\overline{P^{rr}Mp}/\overline{P^{rr}Mp}$, designated "homozygous variegated." Genotypes 1 and 2 are alike in possessing a single variegated allele, but genotype 2 carries a hemizygous transposed Modulator also. Genotypes 2 and 3 differ in having one and two variegated alleles, respectively, and each has two doses of Mp. Genotypes 1 and 3 are heterozygous and homozygous for the variegated allele, neither possessing a transposed Modulator.

The ears borne by these three classes of plants are illustrated in Figure 1. It is seen that the three phenotypes are readily distinguishable, and, of course, they differ in the character of their offspring in testcrosses with P^{wr}.

The light variegated phenotype is represented in Table 2 by two families. The transposed Modulators carried in these families resulted from independent mutations of P^{vv}. The significance of the fact that the frequency of mutation of P^{vv} to P^{rr} in the two cases is closely comparable will be discussed later.

Medium variegated, light variegated, and homozygous variegated ears from the converted W23 inbred stock were scored for mutations to self color. Size of the mutant area was recorded, and the results were summarized in terms of number of

red stripes or patches per 1,000 kernels. Size of stripe was determined with the aid of models. The latter were constructed in accordance with the findings of Anderson and Brink[5] that size is a function both of time when the stripe was initiated and position on the kernel. The models were obtained by superimposing a circle, with its center on the point of silk scar and a diameter parallel to the germinal face, upon scale drawings of a kernel as viewed from the top. The circle was then divided into eight equal sectors. The length of the included arc of sectors falling on the abgerminal face of the kernel was greater than those falling on the germinal face because the kernel develops asymmetrically in relation to the point of silk attachment. Stripes covering one segment were called one-eighth-kernel mutations and were the smallest scored. Stripes traversing two sectors were scored as mutations covering one-fourth of the kernel. Additional size classes of mutants recorded were those covering one-half kernel, one kernel, and two or more kernels.

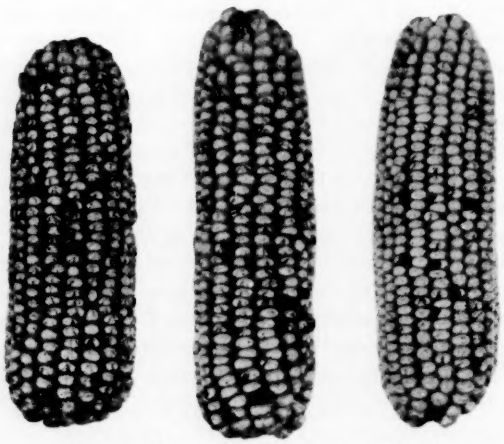

FIG. 1.—Three genotypes of variegated pericarp in maize. *Left to right:* medium variegated (P^{vv}/P^{vr}), homozygous variegated (P^{vv}/P^{vv}), and light variegated (P^{vv}/P^{vr} + tr $Mp/-$).

The mutations were distributed into five size classes, as the stripes did not always fall on the sectors described. These classes were as follows: one-eighth to less than one-fourth, one-fourth to less than one-half, one-half to less than one, one kernel, and two or more kernels. The classes are referred to by their minimal values rather than by their mid-points because the minimal values are in geometric progression with the one-kernel class, which has no range. Mutant stripes covering less than one-eighth of a kernel, although frequent in medium variegateds particularly, are difficult to score and so were not recorded.

Experimental Results.—The distributions of offspring in the families from which representative ears were scored for somatic mutations to self color are shown in Table 1. The phenotypic grouping of the variegateds is somewhat arbitrary, since the "light" ears in one class of families, although similar, did not correspond

precisely with those in another. "Very lights" are mutants of a special type[4] not relevant to the present experiment. These data demonstrate the homogeneous character of the stocks used, the regular distribution of the P^{vv} allele, and the transmission of transposed Modulator independently of the P locus.

TABLE 1

DISTRIBUTION OF OFFSPRING OF DIFFERENT GENOTYPES ACCORDING TO CLASS OF VARIEGATION

GENOTYPE VARIEGATED PARENT	PEDIGREE NO.	COLORED OFFSPRING				COLORLESS PERICARP, RED COB	TOTAL
		Very Light	Light	Medium	Self Colored		
P^{vv}/P^{wr}	6A-40-8⊗	5	20	...	...	...	25*
P^{vv}/P^{wr} + tr $Mp/-$	6-291-1 × W23	1	19	62	20	54	156†
P^{vv}/P^{vv}	Bk 293-1⊗	2	24	0	2	0	28

* P^{vv}/P^{vv} offspring.

† P^{vv}/P^{wr} and P^{wr}/P^{wr} offspring.

The number of mutations per 1,000 kernels for medium variegateds, light variegateds, and homozygous variegateds and their distribution according to stripe size are shown in Table 2. Differences between families within a given variegated

TABLE 2

DISTRIBUTION OF NUMBER OF MUTATIONS OF P^{vv} TO P^{rr} PER 1,000 KERNELS

GENOTYPE OF EAR SCORED	PEDIGREE NO.	ESTIMATED TOTAL KERNELS	NO. EARS SCORED	NO. MUTATIONS PER 1,000 KERNELS					TOTAL
				2 or More	1	1–0.5	0.5–0.25	0.25–0.125	
P^{vv}/P^{wr}	6-291-1×W23	4,857	10	0.62	8.65	16.68	25.12	39.12	...
	6A-40-8⊗	4,351	8	.92	9.42	15.63	24.59	32.64	...
	Total	9,208	18	.76	9.01	16.18	24.87	36.06	86.88
P^{vv}/P^{vv}	Bk 293-1⊗	9,295	16	.86	7.10	13.77	20.33	22.06	...
	6A-40-8×	9,736	16	.31	6.37	7.91	12.74	16.54	...
	Total	19,031	32	.58	6.73	10.77	16.45	19.23	53.76
P^{vv}/P^{wr} + tr $Mp/-$	6-291-1×W23	5,466	10	.28	3.48	6.95	8.23	10.24	...
	6A-40-8⊗	10,344	17	.10	2.51	4.25	8.41	9.76	...
	Total	15,810	27	0.13	2.85	5.19	8.35	9.93	26.45

genotype appear to be due to fluctuation around a common mean and so may be combined in the analysis. The values plotted in Figure 2 are based upon the total mutations in each variegated genotype.

The frequency of somatic mutation to self color in medium variegateds (P^{vv}/P^{wr}) proved to be about 1.6 times as high as that in homozygous variegateds in these stocks. Although the observed difference is of the same order as that originally reported by Emerson[2] for these two genotypes, the magnitude, as such, of the difference is not important. As pointed out in the introductory paragraph, the amount of the difference may be expected to vary, and does in fact vary, from one strain to another, depending both upon the particular P^{vv} allele concerned and the genotypic background. On the other hand, with a given P^{vv} allele on a given genetic background, there should be a constant relation (2:1) between the mutation rates in homozygous variegateds and light variegateds if Modulator dosage is the controlling factor.

As the data in Table 2 and Figure 2 show, mutations of P^{vv} to P^{rr}, in fact, are almost exactly twice as frequent in homozygous variegateds (P^{vv}/P^{vv}) as in light variegateds (P^{vv}/P^{wr} + tr $Mp/-$). The chi-square test shows a close fit on this

basis for each of the kernel-stripe classes which can be compared. Further confirmation of the relationship is the fact that the ratio of the regression coefficients of the mutation frequencies for the homozygous variegated and light variegateds was 1.87, a value which is not significantly different from 2.0, according to the *t*-test.

Discussion.—A premise implicit in the design of this experiment was that transposition of Modulator from the *P* locus to a position elsewhere in the genome is not necessarily accompanied by a change in state (mutation) of Modulator. The validity of this assumption is borne out by the fact that the mutation rates of P^{vv} to P^{rr} in the two light variegated families scored, 6-291-1 and 6A-40-8, carrying Modulators independently transposed from the *P* locus to unknown but almost certainly different sites, were essentially the same. Inspection of the ears based on two additional light variegated mutations within the W23 inbred stock showed that they also were alike and indistinguishable in phenotype from the particular light variegateds used in the present experiment. This evidence supports the view that the modifying effect of Modulator on P^{vv} to P^{rr} mutations is, in fact, independent of the location of *Mp* in the genome.

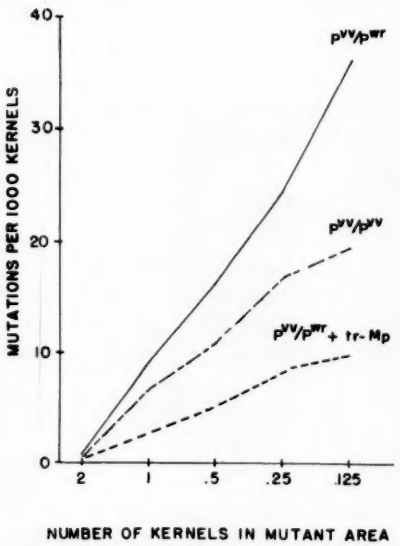

FIG. 2.—Number of mutations of P^{vv} to P^{rr} per 1,000 kernels, distributed according to size of the mutant area. The abscissa is on a geometric scale, which gives an approximately linear relation.

It would be expected, therefore, that *Mp* as a component of a P^{vv} allele would have the same action on mutation of a second P^{vv} allele in the nucleus as a transposed Modulator. The data presented in Table 2 fully substantiate this expectation. The total number of P^{vv} to P^{rr} mutations of all sizes exceeding one-eighth kernel on the P^{vv}/P^{vv} ears is 53.76 per 1,000 kernels. The corresponding value for the light variegateds (P^{vv}/P^{wr} + tr *Mp*/−) is 26.45, or almost exactly one-half. Both genotypes have one Modulator in the genome, in addition to the one directly associated with the mutating allele. In other words, a Modulator at one allele acts as a transposed *Mp* with respect to the other. The validity of this relationship is borne out by the further fact that, within sampling limits, the distribution of the mutations between the different stripe-size classes in the two arrays also conforms to expectation on this basis.

It is interesting to recall at this point the properties of the system which Rhoades[6] described controlling mutations of a_1 to A_1 in the presence of Dotted (*Dt*) in maize. Rhoades found that the number of such mutations was influenced by dosage of both a_1 and *Dt*. The frequency of mutation rose linearly with the dosage of a_1 but at an accelerating rate as the number of *Dt* factors was varied from one to three.

Thus both *Dt* and *Mp* are nonlinear in their respective actions, but the dosage effects are of opposite direction.

Summary.—The effect of Modulator on stability of the variegated pericarp allele (P^{vv} or $P^{rr}Mp$) in somatic tissue was studied in three otherwise near-isogenic genotypes designated "medium variegated" ($\overline{P^{rr}Mp/P^{wr}}$), "light variegated" ($\overline{P^{rr}Mp/P^{wr}}$ + tr $Mp/-$), and "homozygous variegated" ($\overline{P^{rr}Mp/P^{rr}Mp}$). As Emerson had earlier observed in unrelated stocks, the single variegated allele in plants heterozygous for a stable *P* gene was found to mutate to self color approximately three times as frequently as either of the alleles in variegated homozygotes. It was postulated that the lower rate in the homozygote was the result of the action of the Modulator component in each variegated allele on mutation to self color of the other variegated allele. The test of this hypothesis which was applied was a comparison of the frequency of mutation in homozygous variegateds, carrying two P^{vv} alleles embodying two Modulator units, and light variegateds, carrying one variegated allele and one transposed Modulator. If the mutation rate of a P^{vv} allele is equally influenced by an additional *Mp* in any position, including the locus of the homologous allele, then twice as many mutations to self color would be expected in the homozygotes as in the light variegateds. The numerical results obtained on scoring the two classes of ears in question for mutations to the self color agreed closely with expectation on this hypothesis.

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ON MAUTNER'S EIGENFUNCTION EXPANSION

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The present note had its origin in a paper of Mautner[1] on the general eigenfunction expansion problem for self-adjoint operators on a Lebesgue space and recent papers of Gårding[2] and Browder[3] which apply Mautner's results to elliptic differential operators.

Let $(S, \sum, \nu)$ be a σ-finite measure space and T be a (possibly unbounded) self-adjoint operator in $L_2(S, \sum, \nu)$. A version of the spectral theorem asserts that

there exist vectors $\{f_\alpha\}$ such that $L_2(S, \sum, \nu)$ is the orthogonal direct sum $\sum H_\alpha$, where H_α is the manifold of all vectors $F(T)f_\alpha$, where $F \in L_2(\mu_\alpha)$, $\mu_\alpha = (E(\cdot)f_\alpha, f_\alpha)$. Moreover, the correspondence $F(T)f_\alpha \to F(\cdot)$ establishes an isometric isomorphism U of H onto the direct sum $\sum L_2(\mu_\alpha)$ which diagonalizes T in the sense that $(UF(T)f)_\alpha(\lambda) = F(\lambda)\,(Uf)_\alpha(\lambda)$ for $f \in D(F(T))$. Following Mautner, we say that T has an *eigenfunction expansion* if for any such diagonalizing decomposition of $L_2(S, \sum, \nu)$ there exist kernels $W_\alpha(s, \lambda)$ such that the corresponding operator U is determined by the equations

$$(Uf)_\alpha(\lambda) = \int_S f(s)\overline{W_\alpha(s, \lambda)}\nu(ds), \tag{1}$$

the integral existing in the mean-square sense[4] in $L_2(\mu_\alpha)$, and the inversion formula

$$f(s) = \sum_\alpha \int_{-\infty}^{\infty} (Uf)_\alpha(\lambda) W_\alpha(s, \lambda)\mu_\alpha(d\lambda) \tag{2}$$

holds, the integrals existing in the mean-square sense[4] in $L_2(S, \sum, \nu)$ and the series converging in the topology of $L_2(S, \sum, \nu)$.

1. *The Main Theorem.*—Our main theorem establishes a sufficient condition that an operator T should possess an eigenfunction expansion. In later sections we discuss the relation of this result to those of Mautner and Gårding. More complete details will appear in a forthcoming book.[5] It should be noted that we assume that $(S, \sum, \nu)$ is σ-finite but do not assume that $L_2(S, \sum, \nu)$ is separable.

1.1 DEFINITION: A bounded linear operator A in $L_2(S, \sum, \nu)$ has property (α) if there exists a sequence $\{S_n\}$ of sets of finite measure covering S such that

$$\nu\text{-ess sup}_{S_n} |(Af)(s)| < \infty, \qquad n = 1, 2, \ldots,$$

for each $f \in L_2(S, \sum, \nu)$. The sequence $\{S_n\}$ of sets will be called a *covering sequence* for the operator A.

If A has property (α), the map $Af \to (Af|S_n)$ of $(Af)(\cdot)$ into its restriction to S_n is a closed and hence bounded linear map of $L_2(S, \sum, \nu)$ into $L_\infty(S_n, \sum, \nu)$, for $n = 1, 2, \ldots$. In fact, this is an equivalent formulation of property (α).

1.2 THEOREM. *Let T be a self-adjoint operator in $L_2(S, \sum, \nu)$ with the resolution of the identity $E(\cdot)$. If for each bounded Borel set e of the real line the projection $E(e)$ has property (α), then T has an eigenfunction expansion.*

1.3. LEMMA. *Under the hypothesis of Theorem* 1.2, *there exists a common covering sequence $\{S_n\}$ for all the projections $E(e)$, i.e., a sequence $\{S_n\}$ such that*

$$\nu\text{-ess sup}_{S_n} |(E(e)f)(s)| < \infty, \qquad n = 1, 2, \ldots,$$

for every bounded Borel set e and $f \in L_2(S, \sum, \nu)$.

Clearly, if $\{C_n\}$ is a suitable covering sequence for $E(e)$ and $e_1 \subseteq e$, it is a suitable covering sequence for $E(e_1)$. Now let θ be a finite positive measure on $(S, \sum)$ such that $\theta(e_0) = 0$ implies $\nu(e_0) = 0$. Let $e_n = [-n, n]$, and select sets $A_n \subseteq S$ such that

$$\nu\text{-ess sup}_{A_n} |(E(e_n)f)(s)| < \infty, \qquad f \in L_2(S, \sum, \nu),$$

while the θ-measure of the complement A_n' of A_n satisfies $\theta(A_n') < 2^{-(n+1)}$. If

$S_1 = \bigcap_{n=1}^{\infty} A_n$, then $\theta(S_1') < 1/2$ and ν-ess sup$_{S_1} |(E(e)f)(s)| < \infty$ for every bounded Borel set e and $f \in L_2(S, \sum, \nu)$. The construction of $S_2, S_3, \ldots$ proceeds by induction.

We now construct the kernels $W_\alpha(s, \lambda)$.

1.4. LEMMA. *Let $\{S_n\}$ be a covering sequence for S such that formula* (3) *holds for every bounded Borel set e and $f \in L_2(S, \sum, \nu)$. Let $g \in L_2(S, \sum, \nu)$, and let $\mu(\cdot) = (E(\cdot)g, g)$. Then there exists a function W defined on the Cartesian product of S and the real line P, measurable with respect to $\nu \times \mu$, such that, if e is any bounded Borel subset of R,*

(i) ν-ess sup$_{s \in S_n} \int_e |W(s, \lambda)|^2 \mu(d\lambda) < \infty$, $n = 1, 2, \ldots$,

(ii) $(E(e)F(T)g)(s) = \int_e W(s, \lambda)F(\lambda)\mu(d\lambda)$, $F \in L_2(\mu)$.

Let $e_n = [-n, n]$. It follows easily that the map A_n: $F(\cdot) \to F(T)g$ is a closed and hence bounded map of $L_2(e_n, \mu)$ into $L_\infty(S_n, \sum, \nu)$. Consequently A_n^* is a bounded map of $L_\infty^*(S_n, \sum, \nu)$ into $L_2(e_n, \mu)$. Now $L_1(S_n, \sum, \nu)$ is isometrically imbedded in $L_\infty^*(S_n, \sum, \nu)$, and the restriction B_n of A_n^* is a bounded map of $L_1(S_n, \sum, \nu)$ into $L_2(e_n, \mu)$. Since $L_2(e_n, \mu)$ is separable, it follows from a theorem of Dunford and Pettis[6] that there exists a $(\nu \times \mu)$-essentially unique $\nu \times \mu$-measurable complex-valued function $W^{(n)}$ defined on $S_n \times e_n$ such that

(iii) $(B_n f)(\lambda) = \int_{S_n} f(s)W^{(n)}(s, \lambda)\nu(ds)$, $f \in L_1(S_n, \sum, \nu)$, $\lambda \in e_n$,

and such that

$$\nu\text{-ess sup}_{S_n} \int_{e_n} |W^{(n)}(s, \lambda)|^2 \mu(d\lambda) = \|B_n\|^2 < \infty.$$

The function W is defined by the formula

$$W(s, \lambda) = W^{(n)}(s, \lambda), \qquad (s, \lambda) \in S_n \times e_n.$$

Formula (ii) is easily established, using Fubini's theorem. *Q.E.D.*

Now let f_α be a family of vectors determining a diagonalizing decomposition of $L_2(S, \sum, \nu)$, and associate with each f_α the corresponding kernel W_α determined by Lemma 1.4. It follows by relatively standard arguments (cf., e.g., Browder, *op. cit.*) that for each $f \in L_2(S, \sum, \nu)$ the integrals $\int_{S_n} f(s)\overline{W_\alpha(s, \lambda)}\nu(ds)$ and $\int_n^n (Uf)_\alpha(\lambda)W_\alpha(s, \lambda)\mu_\alpha(d\lambda)$ exist for almost all λ and s, respectively. Moreover,

$$(Uf)_\alpha(\lambda) = \lim_{n \to \infty} \int_{S_n} f(s)\overline{W_\alpha(s, \lambda)}\nu(ds), \; f \in L_2(S, \sum, \nu), \tag{4}$$

and

$$(P_\alpha f)(s) = \lim_{n \to \infty} \int_{-n}^{n} (Uf)_\alpha(\lambda)W_\alpha(s, \lambda)\mu_\alpha(d\lambda), \quad f \in L_2(S, \sum, \nu), \tag{5}$$

where P_α is the perpendicular projection of $L_2(S, \sum, \nu)$ onto H_α, the limits being in the topology of $L_2(\mu_\alpha)$ and $L_2(S, \sum, \nu)$, respectively. Formula (2) follows from the fact that $f = \sum_\alpha P_\alpha f$.

1.5. COROLLARY. *A self-adjoint operator T in $L_2(S, \sum, \nu)$ has an eigenfunction expansion if there exists a sequence $\{S_n\}$ of sets of finite ν-measure covering S such that every f in $\bigcap_{m=1}^{\infty} D(T^m)$ is ν-essentially bounded on each of the sets S_n.*

This useful corollary follows from the observation that $E(e)L_2(S, \sum, \nu) \subset D(T^m)$ for every m when e is bounded.

Mautner[1] showed that if, for some λ, the resolvent $R(\lambda; T)$ of a self-adjoint operator T in $L_2(S, \sum, \nu)$ was an integral operator of Carleman type (i.e.,

$$(R(\lambda; T)f)(s) = \int_S K(s, t)f(t)\nu(dt),$$

where K is product-measurable and $K(s, \cdot) \in L_2(S, \sum, \nu)$ for almost all s), then T has an eigenfunction expansion. Since the range of $R(\lambda; T)$ is $D(T)$, the domain of T, it follows immediately from Mautner's hypothesis that for each $g = R(\lambda; T)f \in D(T)$ we have

$$|g(s)| \leq |f|\theta(s),$$

where $\theta(s) = [\int_S |K(s, t)|^2\nu(dt)]^{1/2}$. On taking $S_n = \{s|\theta(s) \leq n\}$, Mautner's result follows from Corollary 1.5. Gårding[2] generalized Mautner's theorem for separable $L_2(S, \sum, \nu)$ to the case that some function $F(T)$ of T is a Carleman operator, where $F(\lambda)$ does not vanish on $\sigma(T)$.[7] In the case that $\inf_{[-n, n]} |F(\lambda)| > 0$, $n = 1, 2, \ldots$, it is easily seen that Gårding's hypothesis implies that each of the projections $E([-n, n])$ has property (α). (Otherwise we obtain hypothesis (α) for all the projections $E(e)$, where $\inf_{\lambda \in e} |F(\lambda)| > 0$, the set $\{\lambda|F(\lambda) = 0\}$ playing the role of the point $\lambda = \infty$ in Theorem 1.2. A straightforward modification of Theorem 1.2 will then yield the more general result of Gårding.)

2. *Elliptic Operators.*—Let S be an open subset of real Euclidean n-space, and let τ be a formally symmetric elliptic differential operator with sufficiently differentiable coefficients. Then τ determines a symmetric operator $T_0(\tau)$ defined on the C^∞ functions whose supports are interior to D. If T is any self-adjoint extension of $T_0(\tau)$, Gårding and Browder prove that an appropriate power of the resolvent of T is a Carleman operator and hence that T has an eigenfunction expansion. The functions $W_\alpha(\cdot, \lambda)$ belong to C^n and $(\tau - \lambda)W_\alpha(\cdot, \lambda) = 0$. Their construction of the Carleman kernel is based on a result of Fritz John which establishes the existence of a local fundamental solution for τ. We shall now indicate how the theorems of Gårding and Browder may be proved simply via our Corollary 1.5, without the introduction of fundamental solutions. We shall say a function $f \in L_2(S, \sum, \nu)$ possesses the partial derivative $\partial f/\partial x^i$ *in the mean-square sense* if

$$\lim_{h \to 0} h^{-1} f(x_1, \ldots, x_i + h, \ldots, x_n) - f(x_1, \ldots, x_i, \ldots, x_n) = \frac{\partial f}{\partial x^i}$$

exists in the topology of $L_2(S, \sum, \nu)$. A function f belongs to the class H_p if it has mean-square partial derivatives of all orders $\leq p$ in each interior subdomain of S. If $p \geq [n/2] + 1$, then, by a result of Sobolev,[8] each f in H_p is continuous. It is known[9] that if m is the order of τ, then $D(T_0(\tau)^*)$, the domain of the adjoint of $T_0(\tau)$, consists of all functions f in H_m such that $\tau f \in L_2(S, \sum, \nu)$, and $T_0(\tau)^* f = \tau f$. Moreover, if $(T_0(\tau)^* f, h) = (g, h)$ for all $h \in D(T_0(\tau))$ and if $g \in H_k$, then $f \in H_{k+m}$. Thus, if T is any self-adjoint extension of $T_0(\tau)$, the functions in $D(T^r)$ $(\subset D((T_0(\tau)^*)^r)$ are continuous if $mr \geq [n/2] + 1$. It follows from Corollary 1.5 that T has an eigenfunction expansion, for we may take for $\{S_n\}$ any sequence of compact sets whose union is S.

In a recent paper L. Hörmander[10] has shown that a certain class of nonelliptic

partial differential operators τ with constant coefficients, called by him *operators of local type*, also have the property that each $f \in \bigcap_{n=1}^{\infty} D((T_0(\tau))^n)$ is continuous. It follows then, by Corollary 1.5, that any self-adjoint extension of $T_0(\tau)$ has an eigenfunction expansion; this was shown by Hörmander, using Gårding's method of proof.

3. *Carleman Operators.*—Our next theorem shows that when $L_2(S, \sum, \nu)$ is separable, the hypotheses for Theorem 1.2 are actually equivalent to the requirement of Gårding that an appropriate function of T should be a Carleman operator.

3.1. THEOREM. *Let T be a self-adjoint operator in a separable space $L_2(S, \sum, \nu)$, and let each projection $E(e)$, bounded, have property (α). Then there exists a bounded function F defined on the real line such that $\inf_{[-n,\, n]} |F(\lambda)| > 0$, $n = 1, 2, \ldots$, and $F(T)$ is an integral operator of Carleman type.*

This result follows from a simple characterization of Carleman operators given in the next lemma. Since it may have independent interest, we state it for a possibly nonseparable space.

3.2 LEMMA. *Let $(S, \sum, \nu)$ be a σ-finite measure space. A bounded operator in $L_2(S, \sum, \nu)$ is of Carleman type if and only if it has a separable range and has property (α).*

Proof: Suppose

$$(Af)(s) = \int_S K(s, t) f(t) \nu(dt), \qquad f \in L_2(S, \sum, \nu),$$

where K is product-measurable and $\theta(s) = \int_S |K(s, t)|^2 \nu(dt) < \infty$ a.e. We have already noted such an operator has property (α). To see that A has a separable range, construct operators A_n with kernels K_n such that $|K_n(s, t)| \leq |K(s, t)|$, $\lim_{n \to \infty} K_n(s, t) = K(s, t)$ $\nu \times \nu$-a.e., and $\iint |K_n(s, t)|^2 \nu(ds) \nu(dt) < \infty$. Then each A_n is a compact operator and hence has a separable range. For $E \in \sum$, define $(P_E f)(s) = f(s) \chi_E(s)$. If $\sup_E \theta(s) < \infty$, we note $|P_E(A_n f - Af)| \to 0$, $f \in L_2(S, \sum, \nu)$. If $E_n = \{s \mid \theta(s) \leq n\}$, then $|Af - P_{E_n} Af| \to 0$, $f \in L_2(S, \sum, \nu)$. From this it follows easily that A has a separable range.

A proof of the sufficiency may be patterned on the proof of Theorem 1.2. Since A has a separable range, we may find a sub σ-field $\overline{\sum}$ of $\sum$ such that $AL_2(S, \sum, \nu) \subset L_2(S, \overline{\sum}, \nu) \subset L_2(S, \sum, \nu)$ and $L_1(S, \overline{\sum}, \nu)$ and $L_2(S, \sum, \nu)$ are separable. The map C_n: $f \to (Af \mid S_n)$ of $L_2(S, \sum, \nu)$ into $L_\infty(S_n, \sum, \nu)$ is closed and bounded. The restriction B_n of C_n^* to $L_1(S_n, \overline{\sum}, \nu)$ is a bounded map into $L_2(S, \sum, \nu)$ having a separable range. Hence, by the theorem of Dunford and Pettis cited above, there exists a $\nu \times \nu$-essentially unique measurable function $K^{(n)}$ defined on $S_n \times S$ such that

$$(B_n f)(t) = \int_{S_n} f(s) K(s, t) \nu(ds), \qquad f \in L_1(S_n, \overline{\sum}, \nu), \quad t \in S,$$

and such that

$$\nu\text{-ess sup}_{s \in S_n} \int_S |K^{(n)}(s, t)|^2 \nu(dt) = \|B_n\|^2 < \infty.$$

The proof may be completed as before, to show that

$$(Af)(s) = \int_S K(s, t)f(t)\nu(dt), \qquad f \in L_2(S, \sum, \nu),$$

where $K(s, t) = K^{(n)}(s, t)$, $(s, t) \in S_n \times S$. *Q.E.D.*

To prove Theorem 3.1, let each projection $E(e)$, e bounded, have property (α), and select a sequence $\{S_n\}$, with the properties of Lemma 1.3, covering S. There exist numbers M_n such that

$$\nu\text{-ess sup } |(E([-n, n])f)(s)| \leq M_n|f|, \qquad s \in S_n.$$

Define $F(\lambda) = \sum_{n=1}^{\infty} M_n^{-1} 2^{-n}\chi_{[-n, n]}(\lambda)$, $\chi_{[-n, n]}$ denoting the characteristic function of the interval $[-n, n]$. Then

$$(F(T)f)(s) = \frac{\sum_{n=1}^{\infty} (E([-n, n])f)(s)}{2^n M_n}, \qquad f \in L_2(S, \sum, \nu),$$

the series converging uniformly on each of the sets S_n. It follows that $F(T)$ has property (α), and thus $F(T)$ is a Carleman operator by Lemma 3.2.

4. *Linear Independence of the Kernels W_α.*—If $L_2(S, \sum, \nu)$ is separable, it is known that there exists a diagonalizing sequence $\{f_n\}$ for T, a finite Borel measure μ on the real line R, and a sequence $R = e_1 \supset e_2 \supset \ldots$ of Borel sets such that $(E(e)f_n, f_n) = \mu(e \cap e_n)$, $n = 1, 2, \ldots$. We shall call the corresponding decomposition an *ordered* decomposition. Let W_i, $i = 1, 2, \ldots$, denote the corresponding kernels whose existence is proved in Lemma 1.4. Then $W_i(s, \lambda)$ may be supposed to vanish for $\lambda \notin e_i$. Since W_i is $\nu \times \mu$-measurable, the function $W_i(\cdot, \lambda)$ is ν-measurable for μ-almost all $\lambda \in e_i$.

4.1. THEOREM. *The ν-measurable functions $W_1(\cdot, \lambda), \ldots, W_n(\cdot, \lambda)$ are linearly independent for μ-almost all λ in e_n.*

The proof proceeds by induction. Suppose that the functions $W_i(\cdot, \lambda)$, $i = 1, \ldots, p - 1$, are linearly independent for μ-almost all λ on e_{p-1}, while the set $W_i(\cdot, \lambda)$, $i = 1, \ldots, p$, is not linearly independent for μ-almost all λ on e_p. By a fairly complicated argument, it is shown there exists a *Borel* subset $\sigma \subset e_p$ of positive μ-measure and *Borel-measurable* functions α_i such that

$$W_p(\cdot, \lambda) = \sum_{i=1}^{p-1} \alpha_i(\lambda)W_i(\cdot, \lambda), \qquad \lambda \in \sigma,$$

the principal tool being a vector form of the well-known theorem of Lusin. By integrating this equation over appropriate sets (cf. Lemma 1.4 [ii]), we may contradict the orthogonality of the manifolds $H_1, \ldots, H_p$. Complete details will be published elsewhere.

We may define the *multiplicity* of a projection $E(e)$ in the resolution of the identity for T as the number of sets e_i such that $\mu(e \cap e_i) \neq 0$ in an ordered decomposition which diagonalizes T. The multiplicity of the identity will also be called the *multiplicity of T*. Let T be a self-adjoint extension of the symmetric operator $T_0(\tau)$ corresponding to an ordinary differential operator τ of order n on an interval I. Theorem 4.1 may be used to show that the multiplicity of T is $\leq n$. The

kernels $W_i(\cdot, \lambda)$ belong to $C^n(I)$ and satisfy the boundary conditions at a fixed end point of I or, more generally, at an end point at which there is a purely discrete spectrum (in a suitable sense). As a consequence we obtain additional information as to the magnitude of the multiplicity of T: e.g., let τ be a real second-order operator on an interval. If the boundary conditions determining T include a boundary condition at a limit-circle end point, then T has multiplicity one.[11]

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[3] F. E. Browder, "Eigenfunction Expansions for Singular Elliptic Operators. I, II," these PROCEEDINGS, **40**, 454–463, 1954. The results are proved for elliptic systems.

[4] The precise sense in which we shall require these integrals to exist is described in formulas (4) and (5).

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ON THE DISTRIBUTION OF CERTAIN FUNCTIONALS OF MARKOFF CHAINS AND PROCESSES

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1. *Functionals of Markoff Chains.*—Let x_n, $n = 0, 1, \ldots$ be a Markoff chain taking on values in an abstract space Ω and having stationary transition probabilities $P_n(x, E) = Pr\{x_{n+k} \in E | x_k = x\}$. Let $P_0(x, E) = \chi_E(x)$, the characteristic function of the set E, and suppose $x_0 = x$, a constant. Let $V(y)$, $y \in \Omega$, be real valued and measurable with respect to $\mathfrak{F}$, the Borel field of sets over which the P_n's are probability measures. In this section we study the distribution of the functional

$$U_n = \sum_{j=0}^{n} V(x_j). \tag{1.1}$$

This distribution will be determined, in principle, if we know the function

$$R(x, E) = \sum_{n=0}^{\infty} E\{e^{i\xi U_n} | x_0 = x, x_n \in E\} P_n(x, E) z^n, \quad 0 \leq z < 1, \xi \text{ real}, \tag{1.2}$$

and Theorem 1 below gives R as the unique solution to Fredholm type equations. Set

$$P_z(x, E) = \sum_{n=0}^{\infty} P_n(x, E) z^n, \qquad 0 \leq z < 1. \tag{1.3}$$

Then we have

THEOREM 1. *The function R of equation (1.2) satisfies uniquely either of the two integral equations*

$$R(x, E) = P_z(x, E) + \int (1 - e^{-i\xi V(y)}) P_z(x, dy) R(y, E), \tag{1.4}$$

$$R(x, E) = P_z(x, E) + \int (1 - e^{-i\xi V(y)}) R(x, dy) P_z(y, E). \tag{1.5}$$

The proof of this theorem is accomplished by integrating the trivial identity

$$e^{i\xi \sum_{j=0}^{n} V(x_j)} = 1 + \sum_{j=0}^{n} (1 - e^{-i\xi V(x_j)}) e^{i\xi \sum_{k=j}^{n} V(x_k)}$$

over the set $x_n \in E$, which has probability $P_n(x, E)$. Then, on multiplying by z^n and summing on $n = 0, 1, \ldots$, we obtain equation (1.4). Equation (1.5) follows similarly by starting with the identity

$$e^{i\xi \sum_{j=0}^{n} V(x_j)} = 1 + \sum_{j=0}^{n} (1 - e^{-i\xi V(x_j)}) e^{i\xi \sum_{k=0}^{j} V(x_k)}.$$

It is simple to show that if R_A satisfies equation (1.4) and R_B equation (1.5), then necessarily $R_A = R_B$, and since the function R of equation (1.2) satisfies both of them, the uniqueness assertion of the theorem follows. Equations (1.4) and (1.5) might be termed the "forward" and "backward" integral equations for R.

We notice that for fixed x, ξ, z, the function R of (1.2) is, as a function of E, a bounded, complex-valued, completely additive measure over $\mathfrak{F}$.

We obtain somewhat more directly the distribution of U_n by putting $E = \Omega$ in equation (1.2). Thus, defining

$$Q(x) = R(x, \Omega) = \sum_{n=0}^{\infty} E\{e^{i\xi U_n} \mid x_0 = x\} z^n,$$

we obtain from equation (1.4) the following equation satisfied by Q:

$$Q(x) = \frac{1}{1 - z} + \int (1 - e^{-i\xi V(y)}) P_z(x, dy) Q(y). \tag{1.6}$$

If $x_n = x + y_1 + y_2 + \ldots + y_n$, $x_0 = x$, where the y_i are independent, identically distributed random variables with common characteristic function $\varphi(t) = E\{\exp(ity_j)\}$, it will follow by taking the Fourier transform of equation (1.5) that

$$\int_{-\infty}^{\infty} e^{ity} R(x, dy) \{e^{-i\xi V(y)} - z\varphi(t)\} = e^{itx}. \tag{1.7}$$

2. *Functionals of Markoff Processes.*—If $x(t)$, $t \geq 0$, is a Markoff process with $x(0) = x$ and $P(x, E, t) = Pr\{x(t + s) \in E \mid x(s) = x\}$ as its stationary transition probability, the results of Section 1 carry over pretty much the same, except that one uses Laplace transforms instead of generating functions. Suppose that for each fixed E, $P(x, E, t)$ is measurable in (x, t) (with respect to $\mathfrak{F}$ and the Lebesgue

sets in $0 \leq t < \infty$) and that $V(y)$ is real, measurable, and such that $\int_0^t E_1^t\{|V(x(t))| \mid x(t) \in E\} < \infty$, $0 \leq t < \infty$, $P(x, E, t) > 0$.

Putting

$$U(t) = \int_0^t V(x(\tau))\, d\tau, \tag{2.1}$$

$$R(x, E) = \int_0^\infty e^{-st} E_1^t\{e^{i\xi U(t)} \mid x(0) = x, x(t) \in E\} \tag{2.2}$$

$$p_s(x, E) = \int_0^\infty e^{-st} P(x, E, t)\, dt, \tag{2.3}$$

for $s > 0$, ξ real, we obtain the following theorem, analogous to Theorem 1:

THEOREM 2. *The function R of equation (2.2) satisfies uniquely either of the two integral equations*

$$R(x, E) = p_s(x, E) + i\xi \int V(y)\, p_s(x, dy)\, R(y, E), \tag{2.4}$$

$$R(x, E) = p_s(x, E) + i\xi \int V(y)\, R(x, dy)\, p_s(y, E). \tag{2.5}$$

The proof is entirely analogous to that of Theorem 1. Equation (2.5) was given by Kac[1] for a symmetric stable process under different conditions and with a different method of proof. Blanc-Lapierre and Fortet[2] gave an analogue of equation (2.5) for a process with nonstationary increments in a somewhat different setting. However, it does not seem to have been noticed that R satisfies the pair of equations (2.4) and (2.5), and it is the interconnection between them (and the corresponding differential equations of the next section) which makes much of the theory simple and cohesive.

Analogous to equation (1.6), we obtain for the function

$$Q(x) = R(x, \Omega) = \int_0^\infty e^{-st} E_1^t\{e^{i\xi U(t)} \mid x(0) = x\}\, dt \tag{2.6}$$

the following integral equation

$$Q(x) = \frac{1}{s} + i\xi \int V(y)\, p_s(x, dy)\, Q(y). \tag{2.7}$$

Theorem 2 shows that to each function V there is associated a unique R. Let now R_1 and R_2 be associated with V_1, V_2. Then it is easily shown that

$$R_2(x, E) - R_1(x, E) = i\xi \int (V_2(y) - V_1(y))\, R_1(x, dy)\, R_2(y, E),$$

a formula which would be useful presumably in a perturbation calculation.

Let $x(t)$ be an additive process with $E_1^t\{\exp(i\xi x(t))\} = e^{t\varphi(\xi)}$. Then, analogous to equation (1.7), we obtain, by taking the Fourier transform of equation (2.4),

$$\int e^{i\sigma y} R(x, dy)(s - i\xi V(y) - \varphi(\sigma)) = e^{i\sigma x}$$

(cf. Kac,[1] who considered the case of a symmetric stable process, in which case $\varphi(\xi) = -|\xi|^\alpha$, $0 < \alpha \leq 2$.)

3. *Differential Equations Satisfied by R.*—Let $\Omega = E^n$, the n-dimensional Euclidean space, and suppose that $P(x, E, t)$ has a density $p(x, y, t)$, $x = (x_1, x_2, \ldots, x_n)$, $y = (y_1, y_2, \ldots, y_n)$ over E^n. Then in many cases $p_s(x, y) = \int_0^\infty e^{-st} p(x, y, t)\, dt$ will be, as a function of x, the negative of the Green's solution to $Lp - sp = 0$, where

$$L = \sum_{i,j} a_{ij} \frac{\partial^2}{\partial x_i \partial x_j} + \sum b_i \frac{\partial}{\partial x_i},$$

which vanishes for $\sum x_i^2 \to \infty$ and is singular at $x = y$, a similar remark holding true for the adjoint equation $L^*p - sp = 0$ as a function of y.

Now if p has this property, it is easy to show that R, considered as a measure over E^n, has a density $r(x, y)$, and a purely formal application of the operator $L - s$ to equation (2.4) and $L^* - s$ to equation (2.5) suggests that r is the negative of the corresponding Green's solution to

$$(L + i\xi V - s)r = 0, \qquad x \neq y,\ V = V(x) \quad (3.1)$$

$$(L^* + i\xi V - s)r = 0, \qquad y \neq x,\ V = V(y). \quad (3.2)$$

It seems difficult to find an existence proof for these differential equations (cf. Rosenblatt[3] for the Wiener process case), but it is possible to show that if p is the *unique* solution to $(L - s)p = 0$, $(L^* - s)p = 0$, then the solution to equations (3.1) and (3.2), if it exists, is unique and is indeed the density corresponding to equation (2.2).

Under similar circumstances the function q corresponding to equation (2.6) satisfies $(L + i\xi V - s)q = -1$, q bounded.

4. *An Illustration.*—Let $x(t)$ be the Uhlenbeck process; i.e., $x(t)$ is Gaussian, mean 0, stationary, Markoffian, with $E(x(t)\, x(t+s)) = \exp(-|s|)$. Put $V(x) = {}^1/_2 \operatorname{sgn} x$, so that $\int_0^t V(x(\tau))\, d\tau + t/2$ is the amount of time $x(\tau)$ is positive, $0 \leq \tau \leq t$. This is the analogue of the so-called "arc sin law" for the Wiener process. We have, namely, for this latter process $Pr\{\int_0^t {}^1/_2 \operatorname{sgn} x(\tau)\, d\tau + t/2 < \alpha\} = (2/\pi) \sin^{-1} \sqrt{\alpha/t}$ (see, e.g., Kac[4]).

In this case, setting $x = 0$, equation (3.2) becomes

$$\frac{d^2r}{dy^2} + \frac{d}{dy}(yr) + ({}^1/_2 i\xi \operatorname{sgn} y - s)r = 0, \qquad y \neq 0.$$

After some tedious calculations the Green's solution can be obtained in terms of Weber functions, and we ultimately obtain

$$\int_{-\infty}^{\infty} r\, dy = q(0) = \int_0^{\infty} e^{-st} E\{e^{i\xi \int_0^t {}^1/_2 \operatorname{sgn} x(\tau) d\tau} \mid x(0) = 0\}\, dt = \frac{s}{s^2 + {}^1/_4\xi^2} +$$

$$\frac{{}^1/_2 i\xi \quad \Gamma({}^1/_2 + s/2 - i\xi/4)\Gamma(s/2 + i\xi/4) - \Gamma({}^1/_2 + s/2 + i\xi/4)\Gamma(s/2 - i\xi/4)}{s^2 + {}^1/_4\, \xi^2\, \Gamma({}^1/_2 + s/2 - i\xi/4)\Gamma(s/2 + i\xi/4) + \Gamma({}^1/_2 + s/2 + i\xi/4)\Gamma(s/2 - i\xi/4)}.$$

In order to get the distribution explicitly, it is necessary to invert this last expression as a Laplace transform on s and a Fourier transform on ξ, which appears very difficult. However, further calculations show that $(1/\sqrt{t}) \int_0^t {}^1/_2 \operatorname{sgn} x(\tau)\, d\tau$ is asymptotically normally distributed with mean 0 and variance ${}^1/_2 \log 2$, $t \to \infty$.

We are preparing for publication some further examples of applied interest.

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COMBINATORIAL PROBLEMS IN THE THEORY OF GRAPHS. III

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1. *Introduction.*—In two previous notes[1] we have presented solutions to a number of counting problems for linear graphs, Note I being devoted to graphs with labeled points and Note II to graphs with unlabeled points. The subject of this note will be the asymptotic behavior of these solutions for graphs in which the number of points is large. For definitions and notation we refer to Notes I and II.

Although the solutions in Notes I and II were given in the form of functional relations between the various counting series, the counting series themselves were not considered as necessarily representing actual functions. Rather, the functional relations were looked upon as a convenient way in which to express the recursion relations between the counting-series coefficients. Certain of the star tree counting series, however, do represent functions analytic in some region about the origin, and in such instances we can use a knowledge of the analytic behavior of the functions to determine the asymptotic behavior of the counting-series coefficients. The methods used in this note are based on this idea and are a generalization of those used by Otter[2] for the case of Cayley trees.

2. *The Asymptotic Numbers for Star Trees with Labeled Points.*—Let $T(p)$ be the number of rooted mixed star trees with p labeled points which are built out of stars from a given collection of types of star. Introducing the counting series

$$T(x) = \sum_{p=1}^{\infty} T(p) \frac{x^p}{p!}, \tag{1}$$

we note that $T(x)$ is equal to $T(x;\ y_1, y_2, \ldots)$, defined by (I, 5), in which each y_σ is set equal to unity, i.e.,

$$T(x) = T(x;\ 1, 1, \ldots). \tag{2}$$

Hence, from the implicit equation (I, 7) satisfied by $T(x;\ y_1, y_2, \ldots)$, we see that $T(x)$ is a solution of the implicit equation

$$F(T, x) \equiv T - x \exp\,[S'(T)] = 0, \tag{3}$$

where $S' \equiv dS/dT$, and

$$S(z) = \sum_{\sigma} \frac{1}{s_\sigma} z^{q_\sigma}, \tag{4}$$

in which q_σ is the number of points and s_σ the symmetry number of a star of type σ, and the sum is over all types of star in the collection. If we for the moment assume that the sum over σ is finite, i.e., the mixed star trees contain only a finite number of types of star, then $F(T, x)$ is an entire function of T and of x. Since $F(T, x)$ is zero for $T = 0$, $x = 0$, while $\partial F/\partial T$ does not vanish for $T = 0$, $x = 0$, it follows from the implicit function theorem that relation (3) may be solved for $T(x)$ which is analytic in some region about $x = 0$. The only singularities of $T(x)$ in the finite plane will be at those values of x for which

$$\frac{\partial F}{\partial T} = 1 - TS'(T) = 0, \tag{5}$$

where we have used relation (3) to eliminate x. In general, there will be many such values of T, but from the fact that $S(z)$ is a polynomial with positive coefficients we can conclude that there is exactly one real positive T, which we denote by T_0, which satisfies equation (5), and that there are no T satisfying equation (5) whose absolute value is less than T_0. Since the coefficients in the power series (1) are all positive, it follows that, if x_0 is the radius of convergence of (1), then $T(x_0) = T_0$. From expression (4) for $S(x)$ we see that $\partial F/\partial T$ will vanish for $T = T_0 \exp(i\theta)$ only if $\theta = 2\pi(m/d)$, $m = 0, 1, \ldots d - 1$, where d is the greatest common divisor of the set of integers $[q_\sigma - 1]$. On the other hand, from the implicit equation (3) we see that

$$T(xe^{i2\pi(m/d)}) = e^{i2\pi(m/d)}T(x), \tag{6}$$

from which it follows that the only singularities of $T(x)$ on the circle of convergence $|x| = x_0$ occur at $x = x_0 \exp[i2\pi(m/d)]$, $m = 0, 1, \ldots, d - 1$.

From equation (6) we see that the behavior of $T(x)$ in the neighborhood of $x = x_0 \exp[i2\pi(m/d)]$ may be expressed in terms of the behavior in the neighborhood of $x = x_0$; hence we confine our attention to the singularity at $x = x_0$. It can readily be shown that

$$\left(\frac{\partial^2 F}{\partial T^2}\right)_{\substack{x = x_0 \\ T = T_0}} = -[S''(T_0) + T_0 S'''(T_0)] \neq 0, \tag{7}$$

and hence the singularity of $T(x)$ at $x = x_0$ must be a branch point of order two. That is, in the neighborhood of $x = x_0$, $T(x)$ is a regular function of $(x_0 - x)^{1/2}$ and may be expanded:

$$T(x) = T_0 - b(x_0 - x)^{1/2} + \ldots. \tag{8}$$

An expression for b may be obtained by differentiating relation (3) with respect to x and then taking the limit as $x \to x_0$. The result is

$$\tfrac{1}{2}x_0 b^2[S''(T_0) + T_0 S'''(T_0)] = T_0. \tag{9}$$

If we let $t(p)$ be the number of free mixed star trees with p labeled points which can be constructed with stars from the given star collection and define the counting series $t(x)$ similarly to equation (1), then we see from (I, 11) that

$$T(x) = x \frac{d}{dx} t(x), \tag{10}$$

or, from (I, 14),

$$t(x) = T(x) - [T(x)S'(T(x)) - S(T(x))]. \tag{11}$$

From these expressions we readily see that, near $x = x_0$,

$$t(x) = t(x_0) - \frac{T_0}{x_0}(x_0 - x) + \frac{2b}{3x_0}(x_0 - x)^{3/2} + \ldots. \tag{12}$$

Using the Cauchy integral formula to express $T(p)$ and $t(p)$ in terms of $T(x)$ and $t(x)$, respectively, and choosing for the contour a circle of radius $r > x_0$ indented around each of the cuts running from the branch points $x_0 \exp[i2\pi(m/d)]$ to infinity, one can show that for large p the main contribution comes from the neighborhood of the branch points. Using equation (6), one sees that the contribution for the branch point $x_0 \exp[i2\pi(m/d)]$ is the same as for the branch point x_0 except for a factor $\exp[i2\pi m(p-1)/d]$. The integral around x_0 is readily evaluated by the method of steepest descent. Adding the contributions of all branch points then yields the following asymptotic expressions for $T(p)$ and $t(p)$:

$$\frac{1}{p!} T(p) \sim \begin{cases} 0, & \dfrac{p-1}{d} \text{ not an integer}, \\ d \dfrac{b}{2\sqrt{\pi}} x_0^{-p+1/2} p^{-3/2}, & \dfrac{p-1}{d} \text{ an integer}, \end{cases} \tag{13}$$

and

$$\frac{1}{p!} t(p) \sim \begin{cases} 0, & \dfrac{p-1}{d} \text{ not an integer}, \\ d \dfrac{b}{2\sqrt{\pi}} x_0^{-p+1/2} p^{-3/2}, & \dfrac{p-1}{d} \text{ an integer}. \end{cases} \tag{14}$$

As a simple application of these results, consider the case of pure Husimi trees in which the stars are m-gons and hence[3]

$$S(z) = \frac{1}{2m} z^m. \tag{15}$$

Using the general results above, we see that

$$T_0 = \left(\frac{2}{m-1}\right)^{1/(m-1)}, \qquad x_0 = \left(\frac{2}{(m-1)e}\right)^{1/(m-1)},$$
$$b^2 = \left(\frac{2}{m-1}\right)^{m/(m-1)} e^{1/(m-1)}, \tag{16}$$

and, of course, $d = m - 1$. In this case an exact expression for $T(p)$ also may be obtained from (I, 8).

$$\frac{1}{p!}\,T(p) = \begin{cases} 0, & n = \dfrac{p-1}{m-1} \text{ not an integer,} \\ \dfrac{p^{n-1}}{2^n n!}, & n = \dfrac{p-1}{m-1} \text{ an integer.} \end{cases} \tag{17}$$

One can readily verify that for large p expression (17) is asymptotically equal to expression (13) when the values (16) are substituted.

A less trivial application is the case of arbitrary mixed Husimi trees. Here the collection of types of star is the collection of all polygons, including the line, so that

$$S(z) = \frac{1}{2}z^2 + \sum_{m=3}^{\infty} \frac{1}{2m}\,z^m. \tag{18}$$

Although $S(z)$ for this case is not a polynomial in z, it can be shown[4] that the discussion above still applies, provided we take T_0 to be the *smallest* real positive value of T satisfying relation (5). After some numerical work, we obtain the results

$$T_0 = 0.45631, \qquad x_0 = 0.23874, \qquad b = 0.87170.$$

In Table 1 the exact and asymptotic expressions for $t(p)$ are compared for $p = 1, \ldots, 6$.

TABLE 1

COMPARISON OF EXACT AND ASYMPTOTIC EXPRESSIONS FOR $t(p)$ FOR MIXED HUSIMI TREES

	p					
	1	2	3	4	5	6
Exact $t(p)$	1	1	4	31	362	5,676
Asymptotic $t(p)$	1	1	3	28	332	5,297

3. *The Asymptotic Distribution of the Points Among the Stars in the Star Trees.*—It is of interest to obtain some measure of the relative importance of a particular type of star in the collection of types of stars used to construct the star trees. One measure which can easily be calculated is the average fraction of the points in the star trees with p labeled points which lie on stars of a given topological type. To accomplish this, we introduce the counting series

$$t_\sigma(x) = (q_\sigma - 1)\left[y_\sigma \frac{\partial}{\partial y_\sigma}\, t(x;\, y_1, y_2, \ldots)\right]_{y_\rho = 1}, \tag{19}$$

i.e., each y_ρ is set equal to unity after the differentiation. From the definition[1] of $t(x;\, y_1, y_2, \ldots)$ it is clear that

$$t_\sigma(x) = \sum_{p=1}^{\infty} \frac{x^p}{p!} \sum_{[n_\rho]}{}' (q_\sigma - 1) n_\sigma\, t(p;\, n_1, n_2, \ldots) \tag{20}$$

is the counting series for the total number of points which lie on stars of type σ in all the star trees with p labeled points.[5] Using expression (I, 14) for $t(x;\, y_1, y_2, \ldots)$ and the implicit equation (I, 7) we readily see that

$$t_\sigma(x) = \frac{q_\sigma - 1}{s_\sigma}\, T^{q_\sigma}(x). \tag{21}$$

Introducing the expansion (8) for $T(x)$, we obtain the following expansion of $t_\sigma(x$ in the neighborhood of $x = x_0$:

$$t_\sigma(x) = t_\sigma(x_0) - \frac{q_\sigma(q_\sigma - 1)}{s_\sigma} T_0^{q_\sigma - 1} b(x_0 - x)^{1/2} + \ldots. \tag{22}$$

We define $f_\sigma(p)$, the average fraction of the points which lie on stars of type σ in the star trees with p labeled points, by[6]

$$f_\sigma(p) = \frac{\text{coeff } x^p: t_\sigma(x)}{(p-1) \text{ coeff } x^p: t(x)}. \tag{23}$$

Asymptotically, using the results of section 2, we see that

$$f_\sigma(p) \sim \frac{q_\sigma(q_\sigma - 1)}{s_\sigma} T_0^{q_\sigma - 1}. \tag{24}$$

If, as will nearly always be the case, $T_0 < 1$, then we see from relation (24) that the distribution of the points among the stars in the star trees is weighted toward the stars with fewer points. As an example, the distribution for the case of arbitrary mixed Husimi trees is given in Table 2.

TABLE 2

ASYMPTOTIC DISTRIBUTION OF THE POINTS AMONG THE STARS IN MIXED HUSIMI TREES

	m						
	2	3	4	5	6	7	8
f_m (per cent)	45.6	20.8	14.2	8.7	5.0	2.7	1.4

4. *The Asymptotic Numbers for Star Trees with Unlabeled Points.*—The discussion in this section will be similar to that in Section 2 for the case of star trees with labeled points. Accordingly, we shall only sketch the arguments and, since the general formulas are rather complicated, restrict our attention to the case of mixed Husimi trees. The generalization to arbitrary mixed star trees is straightforward.

Let $\Theta(p)$ be the number of rooted mixed Husimi trees with p labeled points. Introducing the counting series

$$\Theta(x) = \sum_{p=1}^{\infty} \Theta(p) x^p, \tag{25}$$

we see that $\Theta(x)/x$ is equal to $\Theta(y_2, y_3, \ldots)$, defined by (II, 11) for the case of Husimi trees, in which each y_m is replaced by x^{m-1}, m being the number of points in an m-gon. That is,

$$\Theta(x) = x\Theta(x, x^2, \ldots, x^{m-1}, \ldots). \tag{26}$$

From the implicit equation (II, 12) satisfied by $\Theta(y_2, y_3, \ldots)$, we see that $\Theta(x)$ is a solution of the implicit equation

$$\Phi(\Theta, x) \equiv \Theta - x \exp\left\{\sum_{k=1}^{\infty} \frac{1}{k} \sum_{m=2}^{\infty} Z'(D_m, \Theta(x^k))\right\} = 0, \tag{27}$$

where $Z(D_m)$ is the cycle index of the dihedral group of order $2m$,[7] $Z'(D_m) = (\partial/\partial f_1)Z(D_m)$, and $Z(D_m, f(x))$ denotes the function of x obtained by replacing the

variable f_s by $f(x^s)$. In relation (27), $\Theta(x^n)$ is considered as a function of x for all $n > 1$, so that $\Phi(\Theta, x)$ is an analytic function of Θ and x for $|\Theta| < 1$ and $|x| \leq \xi_0 < 1$, where ξ_0 is the radius of convergence of the power series (25).[8] In order to show that ξ_0 is greater than zero, we note that

$$\text{coeff } x^n\colon Z'(D_m, \Theta(x^k)) \leq \text{coeff } x^n\colon \Theta^{k(m-1)}(x), \tag{28}$$

and, therefore, the power series (25) is bounded term by term by the power-series expansion of the function $\bar{\Theta}(x)$ which is a solution of the implicit equation

$$\bar{\Phi}(\Theta, x) \equiv \Theta - x \prod_{m=2}^{\infty} \frac{1}{1 - \Theta^{m-1}} = 0. \tag{29}$$

Since the infinite product converges for $|\bar{\Theta}| < 1$, and since $\bar{\Phi} = 0$ while $\partial\bar{\Phi}/\partial\bar{\Theta} = 1$ when $\bar{\Theta} = 0$ and $x = 0$, it follows from the implicit function theorem that the radius of convergence of $\bar{\Theta}(x)$, which is less than or equal to ξ_0, is greater than zero. Hence ξ_0 is greater than zero.

The function $\Theta(x)$ must have a singularity on the circle of convergence at $x = \xi_0$, from which it follows that[9]

$$\left(\frac{\partial\Phi}{\partial\Theta}\right)_{\substack{x=\xi_0 \\ \Theta=\Theta(\xi_0)}} = 1 - \Theta(\xi_0) \sum_{m=2}^{\infty} Z''(D_m, \Theta(\xi_0)) = 0. \tag{30}$$

Equations (30) and (27) together determine ξ_0 and $\Theta(\xi_0)$. Next, one can show that $\partial^2\Phi/\partial\Theta^2 \neq 0$ for $x = \xi_0$ and $\Theta = \Theta(\xi_0)$, and hence the singularity of $\Theta(x)$ at $x = \xi_0$ is a branch point of order 2. That is, near $x = \xi_0$,

$$\Theta(x) = \Theta(\xi_0) - \beta(\xi_0 - x)^{1/2} + \ldots. \tag{31}$$

An expression for β may be obtained by differentiating relation (27) with respect to x and taking the limit as $x \to \xi_0$. The result is

$$\tfrac{1}{2}\beta^2\xi_0 \sum_{m=2}^{\infty} [Z''(D_m, \Theta(\xi_0)) + \Theta(\xi_0)Z'''(D_m, \Theta(\xi_0))] = \Theta(\xi_0)\Bigg[1 + \sum_{k=2}^{\infty}\sum_{m=2}^{\infty} \xi_0{}^k\dot{\Theta}(\xi_0{}^k) \times Z''(D_m, \Theta(\xi_0{}^k)) + 2\sum_{k=1}^{\infty}\sum_{m=2}^{\infty} \xi_0{}^{2k}(\xi_0\dot{\Theta}^{2k}) \frac{\partial}{\partial f_2} Z'(D_m, \Theta(\xi_0{}^k))\Bigg], \tag{32}$$

where $\dot{\Theta} \equiv d\Theta/dx$.

If $\theta(p)$ is the number of free mixed Husimi trees with p unlabeled points and $\theta(x)$ is the corresponding counting series, then $\theta(x)$ may be expressed in terms of $\Theta(x)$ by means of (II, 19):

$$\theta(x) = \Theta(x) - \sum_{m=2}^{\infty} [\Theta(x)Z'(D_m, \Theta(x)) - Z(D_m, \Theta(x))]. \tag{33}$$

Expanding both sides of equation (33) about $x = \xi_0$, we see that, because of equation (30), the coefficient of $(\xi_0 - x)^{1/2}$ vanishes, and we obtain

$$\theta(x) = \theta(\xi_0) - \gamma(\xi_0 - x) + \delta(\xi_0 - x)^{3/2} + \ldots, \tag{34}$$

where

$$\delta = \frac{2\beta}{3\xi_0}\left[1 + \sum_{k=2}^{\infty}\sum_{m=2}^{\infty} \xi_0^k \dot{\Theta}(\xi_0^k) Z''(D_m, \Theta(\xi_0^k)) + 2\sum_{k=1}^{\infty}\sum_{m=2}^{\infty} \xi_0^{2k}\dot{\Theta}(\xi_0^{2k}) \frac{\partial}{\partial f_2} Z'(D_m, \Theta(\xi_0^k))\right]. \quad (35)$$

The asymptotic expressions for $\theta(p)$ and $\Theta(p)$ are obtained exactly as in Section 2. The results are

$$\Theta(p) \sim \frac{\beta}{2\sqrt{\pi}} \xi_0^{-p+1/2} p^{-3/2}, \quad (36)$$

$$\theta(p) \sim \frac{3\delta}{4\sqrt{\pi}} \xi_0^{-p+3/2} p^{-5/2}. \quad (37)$$

The numerical values are as follows:

$$\xi_0 = 0.22215, \qquad \Theta(\xi_0) = 0.45381, \qquad \beta = 4.395, \qquad \delta = 11.46. \quad (38)$$

In Table 3 the exact and asymptotic expressions for $\Theta(p)$ and $\theta(p)$ are compared for $p = 1, \ldots, 8$.

TABLE 3

COMPARISON OF EXACT AND ASYMPTOTIC EXPRESSIONS FOR $\Theta(p)$ AND $\theta(p)$ FOR MIXED HUSIMI TREES

	p							
	1	2	3	4	5	6	7	8
Exact $\Theta(p)$	1	1	3	8	26	84	297	1,066
Asympt. $\Theta(p)$	1	1	3	9	30	102	365	1,347
Exact $\theta(p)$	1	1	2	4	9	23	63	188
Asympt. $\theta(p)$	1	1	1	2	5	15	45	146

[1] G. W. Ford and G. E. Uhlenbeck, "Combinatorial Problems in the Theory of Graphs. I," these PROCEEDINGS, **42,** 122, 1956; G. W. Ford, R. Z. Norman, and G. E. Uhlenbeck, "Combinatorial Problems in the Theory of Graphs. II," these PROCEEDINGS, **42,** 203, 1956. We shall refer to these as "Note I" and "Note II," respectively, and equations in them will be referred to as "(I, 17)," etc.

[2] R. Otter, "The Number of Trees," *Ann. Math.*, **49,** 583, 1948.

[3] The case $m = 2$, Cayley trees, is an exception. For Cayley trees $S(z) = z^2/2$, $T_0 = 1$, $x_0 = e^{-1}$, and $b^2 = 2e$.

[4] The conditions on $S(z)$ for which the discussion of Sec. 2 applies will be considered in the next note in this series.

[5] There is a certain arbitrariness as to whether the number of points associated with a star of type σ *in a star tree* is q_σ or $q_\sigma - 1$. The choice here is the more convenient.

[6] Note that, due to relation (I, 6), $\sum_\sigma f_\sigma(p) = 1$.

[7] To be specific:

$$Z(D_m) = \frac{1}{2m}\sum_{s|m}{}' \varphi(s) f_s^{m/s} + \begin{cases} \frac{1}{4}(f_1^2 f_2^{(m-2)/2} + f_2^{m/2}), & m \text{ even}, \\ \frac{1}{2} f_1 f_2^{(m-1)/2}, & m \text{ odd}, \end{cases}$$

where $\varphi(s)$ is the number of integers less than s and relatively prime to s; $\varphi(1) \equiv 1$.

[8] Clearly $\xi_0 < 1$, since the counting series $T(x)$ for graphs of labeled points is bounded by $\Theta(x)$, so that $\xi_0 \leq x_0 < 1$. That $\Theta(p) \geq T(p)/p!$ follows from the fact that to every graph with unlabeled points there correspond s graphs with labeled points, where s is the number of symmetries of the graph.

[9] The primes on Z always refer to differentiations after f_1.

*COMPATIBLE SEMINORMS IN A VECTOR LATTICE**

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Let X be a vector space. A locally convex topology τ in X is given by a system of seminorms p_α, $\alpha \epsilon A$, for which X_τ is a Hausdorff space. We suppose that there is an order relation ω in X for which X_ω is a vector lattice. We suppose, further, that the order dual, X_ω', which consists of all bounded linear forms on X_ω, is a total subspace of the algebraic dual X' of X, i.e., for every $x \neq 0$ there is a bounded f such that $f(x) \neq 0$. In particular, this implies that X_ω is Archimedean. A seminorm p is compatible with ω if $|x| \geqq |y|$ implies $p(x) \geqq p(y)$. A locally convex topology τ is compatible with ω if its seminorms may be chosen so as to be compatible with ω. For general information see N. Bourbaki, *Éléments de mathématique.*[1]

Under the above assumptions, we observe that for every ω there is a finest τ compatible with ω. In particular, we show that there is at most one Banach topology compatible with ω, and, if it exists, it is the finest locally convex topology compatible with ω. Thus, for various function spaces, the norm in use is seen to yield the only possible Banach space compatible with the natural order relation of the space.

We first note that if p is a seminorm compatible with ω, then the continuity seminorm set of p is contained in X_ω'. For, if $f(x)$ is a linear form on X such that, for some M, $|f(x)| \leqq Mp(x)$, for all $x \epsilon X$, then, for every $y > 0$, $|z| \leqq y$ implies $p(z) \leqq p(y)$, so that

$$|f(z)| \leqq Mp(z) \leqq Mp(y),$$

which means that $f(x)$ is bounded. We thus have the fact that if τ is compatible with ω, then $X_\tau' \subset X_\omega'$.

We next note that there is a finest convex topology τ compatible with ω, namely, the one given by all the compatible seminorms. We point out that for this topology $X_\tau' = X_\omega'$. For, if $f(x) \epsilon X_\omega'$ is positive, the function $p(x) = f(|x|)$ is a seminorm compatible with ω for which $f(x)$ is in the continuity seminorm set of $p(x)$. But every bounded linear form on X_ω is the difference of two positive linear forms. Hence, for the finest topology τ compatible with ω, we have $X_\tau' \supset X_\omega'$, so that $X_\tau' = X_\omega'$. For a total subspace $Y \subset X'$, we call the finest locally convex space X_τ for which $X_\tau' = Y$ a *Mackey space*, and summarize the above remarks in

Theorem 1. *If τ is compatible with ω, then $X_\tau' \subset X_\omega'$. If τ is the finest convex topology compatible with ω, then $X_\tau' = X_\omega'$. If τ is compatible with ω, X_τ is a Mackey space, and $X_\tau' = X_\omega'$, then τ is the finest convex topology compatible with ω.*

In applying this theorem to Banach spaces, we first prove

Lemma 1. *If X_ω is a vector lattice and τ: $\|x\|$ is a compatible norm for which X_τ is a Banach space, then, if $\{x_n\}$ is a Cauchy sequence of increasing positive elements, $x = \lim_{n \to \infty} x_n$ implies $x \geqq x_n$ for every n.*

Proof: Suppose that $(x_n - x)^+ > 0$ for some n. Then $(x_p - x)^+ \geqq (x_n - x)^+$ for every $p > n$. But

$$|x_p - x| \geqq (x_p - x)^+,$$

so that

$$\|x_p - x\| \geqq \|(x_n - x)^+\|.$$

Since $\|(x_n - x^+\| > 0$, it follows that $\{x_p\}$ does not converge to x.

THEOREM 2. *If X_ω is a vector lattice and τ: $\|x\|$ is a compatible norm for which X_τ is a Banach space, then it is the finest convex topology compatible with X_ω. Thus a vector lattice may be normed as a Banach lattice in at most one way.*

Proof: Since X_τ is a Mackey space and $X_\tau' \subset X_\omega'$, we need only show that every positive $f(x)$ on X_ω belongs to X_τ'. Suppose, then, that $f(x)$ is not continuous but is positive. Then, for every n, there is an x for which $\|x\| \leqq 1$ and $|f(x)| \geqq n$. Since, by compatibility, $\||x|\| = \|x\|$ and since

$$(f|x|) = f(x^+ + x^-) \geqq |f(x^+ - x^-)| = |f(x)|,$$

it follows that there is an $x > 0$ for which $\|x\| \leqq 1$ and $|f(x)| \geqq n$. Now let $\{x_n\}$ be a sequence of positive elements such that, for every n,

$$\|x_n\| \leqq 1 \qquad \text{and} \qquad |f(x_n)| \geqq 2^{2^n}.$$

Let

$$y_n = \frac{1}{2}x_1 + \frac{1}{2^2}x_2 + \ldots + \frac{1}{2^n}x^n.$$

Then $\{y_n\}$ is an increasing sequence of positive elements. It is a Cauchy sequence since

$$\|y_{n+p} - y_n\| = \left\|\frac{1}{2^{n+1}}x_{n+1} + \ldots + \frac{1}{2^{n+p}}x_{n+p}\right\| < \frac{1}{2^n},$$

for every n, p. Hence, if $y = \lim_{n \to \infty} y_n$, then, by the lemma, $y \geqq y_n$, so that

$$f(y) > f(y_n) > \frac{1}{2^n}f(x_n) \geqq 2^n$$

for every n, which is impossible.

For all the spaces of real functions and sequences of analysis the order relation is the one for which the positive elements are those for which $f(x) \geqq 0$ everywhere, or almost everywhere, as the case may be, or $x_n \geqq 0$ for all n. As examples of Theorem 2, we mention only the two most important cases.

COROLLARY 1. *For the vector lattice of continuous functions on a compact set S the only compatible Banach space topology is given by*

$$\|f\| = \max_{x \in S} |f(x)|.$$

COROLLARY 2. *For the vector lattice L_p of pth-power integrable functions, $p \geqq 1$, on a measure space S, the only compatible Banach space topology is given by*

$$\|f\| = [\int_S |f(x)|^p d\mu]^{1/p}.$$

There are important vector lattices whose associated finest compatible convex topology is not a Banach space topology, and we hope to be able to discuss the general situation in the future.

* Supported by National Science Foundation Grant NSF G-2267.

[1] N. Bourbaki, *Éléments de mathématique;* Livre V: *Espaces vectoriels topologiques,* chaps. ii, iii, iv, Paris, 1953; Livre VI: *Intégration,* chap. ii, Paris, 1952.

A FORMULA FOR SEMISIMPLE LIE GROUPS

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In order to save space we shall follow strictly the notation of an earlier paper.[1] Define $\mathfrak{k}_0$, $\mathfrak{p}_0$ as usual,[2] and suppose that $\mathfrak{h}_0 = \mathfrak{h}_0 \cap \mathfrak{k}_0 + \mathfrak{h}_0 \cap \mathfrak{p}_0$. Then we say that $\mathfrak{h}_0$ is fundamental if $\mathfrak{h}_0 \cap \mathfrak{k}_0$ is maximal Abelian in $\mathfrak{k}_0$. Any two fundamental Cartan subalgebras of $\mathfrak{g}_0$ are conjugate under G_0.

THEOREM 1. *Let $\mathfrak{h}_1$ be a connected component of $\mathfrak{h}_0'$. Then there exists a real number c such that*

$$\lim_{H \to 0} F_f(H; \partial(\pi)) = cf(0) \qquad (H \in \mathfrak{h}_1)$$

for every $f \in \mathcal{C}(\mathfrak{g}_0)$. Moreover, $c = 0$ if $\mathfrak{h}_0$ is not fundamental.

THEOREM 2. *Suppose that $\mathfrak{h}_0$ is fundamental and $\mathfrak{h}_1, \ldots, \mathfrak{h}_r$ are all the distinct connected components of $\mathfrak{h}_0'$. Let c_j be the real number of Theorem 1 corresponding to $\mathfrak{h}_j$ $(1 \leq j \leq r)$. Then $c_1 + c_2 + \ldots + c_r \neq 0$.*

The following result plays an essential role in the proofs of the above theorems. Let dH denote the Euclidean measure on $\mathfrak{h}_0$ and dk the Haar measure on the compact analytic subgroup K_0 of G_0 corresponding the $\mathfrak{k}_0$.

LEMMA 1. *Assume that $\mathfrak{h}_0 \subset \mathfrak{k}_0$, and for any $g \in \mathcal{C}(\mathfrak{h}_0)$ put*

$$\hat{g}(X) = \int_{K_0 \times \mathfrak{h}_0} \exp(iB(X, kH))\pi(H)^2 \sum_{s \in W} g(sH)\, dk\, dH \qquad (X \in \mathfrak{g}_0)$$

Then the integral

$$F_{\hat{g}}(H) = \pi(H) \int_{G^*} \hat{g}(x^*H) dx^*$$

is convergent for $H \in \mathfrak{h}_0'$, and there exists a constant $c \neq 0$ such that

$$\sum_{s \in W} \epsilon(s) F_{\hat{g}}(sH') = c \int_{\mathfrak{h}_0} \sum_{s \in W} \epsilon(s) \exp(iB(H', sH))\pi(H) g(H)\, dH$$

for all $H' \in \mathfrak{h}_0'$ and $g \in \mathcal{C}(\mathfrak{h}_0)$.

Let G be any connected Lie group whose Lie algebra is $\mathfrak{g}_0$, and let A be the Cartan subgroup of G corresponding to $\mathfrak{h}_0$.

LEMMA 2. *Let Ξ be the centralizer in G of an element $a_0 \in A$. Denote the natural mapping of G on $\bar{G} = G/\Xi$ by $x \to \bar{x}$ $(x \in G)$. Then we can find a neighborhood B of a_0 in A with the following property. Given any compact set ω in G, there exists a compact set Ω in G satisfying the condition that if $xax^{-1} \in \omega$ for some $a \in B$ and $x \in G$, then $\bar{x} \in \bar{\Omega}$.*

By combining this with Theorem 2 of Paper 1, we can obtain certain results on G as follows. λ being an indeterminate, let $D(x)$ $(x \in G)$ denote the coefficient of λ^l in $\det\{(\lambda + 1)I - \mathrm{Ad}(x)\}$, where l is the rank and I the identity mapping of $\mathfrak{g}$. Let A' be the set of those points $a \in A$ where $D(a) \neq 0$. We regard A' as an open submanifold of the Lie group A. Note that $G/A = G_0/A_0 = G^*$, and put $a^{x^*} = xax^{-1}$ $(a \in A, x \in G)$, where x^* denotes the coset xA in G^*. Let $C_c^\infty(G)$ be the set of all indefinitely differentiable functions on G which vanish outside a compact set. Then the integral

$$\phi_f(a) = |D(a)|^{1/2} \int_{G^*} f(a^{x^*})\, dx^*$$

converges for $f \in C_c^\infty(G)$ and $a \in A'$, and ϕ_f is of class C^∞ on A'. Let $\mathfrak{B}$ be the universal enveloping algebra of $\mathfrak{g}$ and $\mathfrak{H}$ the subalgebra of $\mathfrak{B}$ generated by $(1, \mathfrak{h})$. Then elements of $\mathfrak{B}$ and $\mathfrak{H}$ can be regarded as differential operators on G and A, respectively.[3] Let $\mathfrak{Z}$ denote the center of $\mathfrak{B}$ and γ the isomorphism of $\mathfrak{Z}$ into $\mathfrak{H}$ described in Paper 2 (p. 394). Then one can show that $\phi_{zf} = \gamma(z)\phi_f$ $(f \in C_c^\infty(G), z \in \mathfrak{Z})$. Let us call a subset of G *bounded* if its closure is compact. Consider the space $\mathcal{C}_0(A')$ of all functions g on A' of class C^∞ satisfying the following two conditions: (1) g is zero (on A') outside some bounded set; (2) $\tau_v(g) = \sup_{a \in A'} |g(a; v)| < \infty$ for every $v \in \mathfrak{H}$. We topologize $C_c^\infty(G)$ in the usual way[4] and $\mathcal{C}_0(A')$ by means of the seminorms τ_v $(v \in \mathfrak{H})$.

THEOREM 3. *$\phi_f \in \mathcal{C}_0(A')$ for $f \in C_c^\infty(G)$ and $f \to \phi_f$ is a continuous mapping of $C_c^\infty(G)$ into $\mathcal{C}_0(A')$. Moreover, for any compact set ω in G we can find a bounded subset B of A' with the following property. If the carrier of f is contained in ω, then $\phi_f = 0$ outside B.*

COROLLARY. *Let da denote the Haar measure on A. Then if g is a measurable function on A which is bounded on every compact set, the mapping T_g: $f \to \int_A g\phi_f da$ $(f \in C_c^\infty(G))$ is a distribution on G.*

The distributions of the form T_g, where g is a character of A, are closely related to the characters of G (see Paper 2).

$S(\mathfrak{h})$ is isomorphic to $\mathfrak{H}$ under a mapping which preserves every element of $\mathfrak{h}$, and so for any $q \in S(\mathfrak{h})$ we get a differential operator on A, which we shall denote by $\partial(q)$. Then Theorems 1 and 2 have the following analogues on G.

THEOREM 4. *Let A_1 be a connected component of A' whose closure contains 1. Then there exists a constant c such that*

$$\lim_{a \to 1} \phi_f(a; \partial(\pi)) = cf(1) \qquad (a \in A_1)$$

for all $f \in C_c^\infty(G)$. Moreover, $c = 0$ if $\mathfrak{h}_0$ is not fundamental.

THEOREM 5. *Now suppose that $\mathfrak{h}_0$ is fundamental and $A_1, \ldots, A_r$ are all the distinct components of A' whose closures contain 1. Let c_j be the constant of Theorem 4 corresponding to A_j $(1 \leq j \leq r)$. Then not every c_j can be zero.*

Theorem 5 gives a simple formula for $f(1)$ in terms of ϕ_f. In case G is compact, the proof of this formula is quite trivial. Moreover, I had proved it earlier[5] for (1) a complex semisimple group and (2) the 2×2 real unimodular group. More recently Gelfand and Graev[6] had extended it to the $n \times n$ real unimodular group.

[1] These PROCEEDINGS, **42**, 252–253, 1956. This will be referred to as "Paper 1."

[2] See *Trans. Am. Math. Soc.*, **75**, 187, 1953.

[3] See *Bull. Am. Math. Soc.*, **61**, 389–396, 1955. This will be referred to as "Paper 2."
[4] See L. Schwartz, *Théorie des distributions*, Vol. 1 (Paris: Hermann & Cie, 1950).
[5] See *Trans. Am. Math. Soc.*, **76**, 514, 1954, and these PROCEEDINGS, **38**, 339, 1952.
[6] *Doklady Akad. Nauk S.S.S.R.*, N.S., **92**, 461–464, 1953.

*ANALYTIC GROUPS OVER COMPLETE FIELDS**

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1. *Introduction.*—If we accept the standpoint that real and complex fields are completions of algebraic number fields by their Archimedean valuations, analytic groups, for instance, over completions by non-Archimedean valuations appear with equal right as real and complex Lie groups. We can see immediately that most of the basic principles of the theory of Lie groups[1] can be carried over to analytic groups over an arbitrary complete field. We shall illustrate this by proving the existence of canonical co-ordinate systems on commutative analytic groups. This is a simple remark, but it is of significance because it contains Mattuck's generalization of Lutz's theorem[2] and the local isomorphism theorem of p-adic additive and multiplicative groups[3] as special cases.

2. *Commutative Analytic Groups.*—By a field we shall understand a field of characteristic 0. A complete field is a field which is complete with respect to the metric topology induced by a real-valued valuation of the field.[4] We observe that fundamental existence theorems, for instance, the existence theorem for a completely integrable system, can be proved by the method of "calcul des limites"[5] over an arbitrary complete field. It was not by chance that the notion of completeness was discovered by Cauchy in proving various existence theorems by this "calcul des limites."

Now, if k is a complete field, analytic manifolds over k, and hence analytic groups over k, can be defined as in the classical case.[6] Also, if $\mathfrak{G}$ is an analytic group of dimension n over k, there are n independent forms of Maurer-Cartan, say $\omega_1, \ldots, \omega_n$, on $\mathfrak{G}$. We know that $\mathfrak{G}$ is commutative if and only if ω_i are closed for $i = 1, \ldots, n$.[7] We shall assume that $\mathfrak{G}$ is commutative.

Pick a system of co-ordinates, say $(x_1, \ldots, x_n)$, on $\mathfrak{G}$ at the neutral element ϵ such that $x_i(\epsilon) = 0$ for $i = 1, \ldots, n$. Then we can express ω_i in terms of the co-ordinates $x_1, \ldots, x_n$ as $\omega_i(x, dx) = \sum_{j=1}^{n} A_{ij}(x)\, dx_j$ for $i = 1, \ldots, n$. Here $A_{ij}(x)$ are convergent power series in the $x_1, \ldots, x_n$, and we have $\det(A_{ij}(0)) \neq 0$. Consider the system of differential equations

$$\frac{\partial u_i}{\partial x_j} = A_{ij}(x)$$

for $i, j = 1, \ldots, n$. Since ω_i are closed, this system is completely integrable. Therefore, u_i can be expressed as convergent power series without constant terms in the $x_1, \ldots, x_n$. Since $\det(A_{ij}(0)) \neq 0$, we can take $(u_1, \ldots, u_n)$ as a system of

co-ordinates on $\mathfrak{G}$ at ϵ. Moreover, in terms of the co-ordinates $u_1, \ldots, u_n$, we have $\omega_i(u, du) = du_i$ for $i = 1, \ldots, n$.

Now pick an element σ_0 of $\mathfrak{G}$ which is very close to ϵ. The functions $u_i(\sigma_0 \cdot \sigma) = v_i(\sigma)$ are defined and analytic in some neighborhood of ϵ; hence there exist convergent power series $f_i(u_1, \ldots, u_n)$ such that $v_i(\sigma) = f_i(u_1(\sigma), \ldots, u_n(\sigma))$ for $i = 1, \ldots, n$. Since ω_i are invariant by translations, we get $\omega_i(v, dv) = \omega_i(u, du)$, i.e., $\partial f_i / \partial u_j = \delta_{ij}$ for $i, j = 1, \ldots, n$. This shows that $f_i(u) = a_i + u_i$ with some constants a_i in k, and we have $u_i(\sigma_0) = v_i(\epsilon) = f_i(0) = a_i$, and hence

$$u_i(\sigma_0 \cdot \sigma) = u_i(\sigma_0) + u_i(\sigma)$$

for $i = 1, \ldots, n$. In other words, the mapping $\sigma \to (u_1(\sigma), \ldots, u_n(\sigma))$ gives an analytic local isomorphism of $\mathfrak{G}$ to the n-dimensional vector group k^n over k. If the valuation of k is Archimedean, we can extend this local isomorphism uniquely to a homomorphism of k^n into $\mathfrak{G}$, and the proof depends only on the Archimedean character of the valuation.

3. *Mattuck's Theorem.*—Let G be an algebraic group variety of dimension n defined over a complete field k. If we denote by $\mathfrak{G}$ the group of rational points of G over k, by the theory of simple points,[8] $\mathfrak{G}$ becomes an analytic group of dimension n over k. Hence, if G is commutative, $\mathfrak{G}$ is locally isomorphic with k^n. In particular, if we take as G the multiplicative group of the universal domain containing k, we see that the multiplicative group of k is locally isomorphic to the additive group of k. On the other hand, if the valuation of k is non-Archimedean, k^n contains small subgroups isomorphic to the n-dimensional vector group over the ring of integers of k. Furthermore, if k is locally compact and nondiscrete, and if G is complete as a variety,[9] $\mathfrak{G}$ is compact; hence, by an elementary theorem on topological groups, any open subgroup is of finite index. Here the compactness of $\mathfrak{G}$ follows from the facts that any complete variety is "dominated" by a projective variety[10] and that the set of rational points over k of a projective variety is compact.

* This work was partially supported by the National Science Foundation.

[1] C. Chevalley, *Theory of Lie Groups*, Vol. 1 (Princeton, N.J.: Princeton University Press, 1946).

[2] A. Mattuck, "Abelian Varieties over P-adic Ground Fields," *Ann. Math.*, **62**, 92–119, 1955.

[3] Cf. C. Chevalley, *Class-Field Theory* (Nagoya University, Japan, 1954), p. 59.

[4] See, e.g., B. L. van der Waerden, *Algebra*, Vol. 1 (Berlin; Springer, 1955), chap. x.

[5] See, e.g., E. Goursat, *A Course in Mathematical Analysis: Differential Equations* (English trans. by Hedrick and Dunkel) (Boston, Mass.: Ginn & Co., 1917), pp. 51–53.

[6] Cf. C. Chevalley, *Theory of Lie Groups*, pp. 68–69, 100.

[7] *Ibid.*, pp. 152–155.

[8] O. Zariski, "The Concept of a Simple Point of an Abstract Algebraic Variety," *Trans. Am. Math. Soc.*, **62**, 1–52, 1947.

[9] A. Weil, *Variétés abéliennes et courbes algébriques* ("Actualités sci. et ind.," No. 1064 [Paris; Hermann & Cie, 1948]), p. 25.

[10] This remark is due to Chow.

ABSTRACT HOMOTOPY. IV

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1. *Introduction.*—The usual definition of the homotopy groups of a simplicial complex involves only its underlying topological space and disregards the simplicial structure. Our main result is the possibility of defining the homotopy groups of a simplicial complex *using only its simplicial structure.* The results will be stated in terms of c.s.s. complexes.[1] They clearly also apply to simplicial complexes, because every simplicial complex may be converted into a c.s.s. complex by a (partial) ordening of its vertices.

The possibility of defining the homotopy groups of a simplicial complex in terms only of its simplicial structure was implicit in the results of a previous note.[2] However, the definition implied by the present results is simpler and resembles the definition of the homology groups.

2. *C.s.s. Groups.*[3]—A *c.s.s. group* G is a c.s.s. complex such that, for every integer $n \geq 0$: (*a*) G_n, the set of its n-simplices, is a group; (*b*) the functions $\epsilon^i\colon G_n \to G_{n-1}$ and $\eta^i\colon G_{n-1} \to G_n$ are homomorphisms (written as operations on the *right*) for $0 \leq i \leq n$.

2.1. *Every c.s.s. group satisfies the extension axiom.*[2]

Let G be a c.s.s. group. Define for each integer $n \geq 0$ a group $\bar{G}_n \subset G_n$ by

$$\bar{G}_n = \bigcap_{i=0}^{n-1} \textit{kernel } \epsilon^i$$

Then $\sigma \in \bar{G}_n$ implies $\sigma\epsilon^n\epsilon \bar{G}_{n-1}$. Let the (right) homomorphism $\bar{\epsilon}^n\colon \bar{G}_n \to \bar{G}_{n-1}$ be defined by $\epsilon^n = \epsilon^n|\bar{G}_n$. Then *image* $\bar{\epsilon}^n$ is a normal subgroup of *kernel* $\bar{\epsilon}^{n-1}$, i.e., $\bar{G} = \{\bar{G}_n, \epsilon^n\}$ is a (not necessarily abelian) chain complex. Its homology groups are $H_n(\bar{G}) = \textit{kernal } \bar{\epsilon}^n/\textit{image } \bar{\epsilon}^{n+1}$.

Let G be a c.s.s. group, and let $1 \in G_0$ be the identity. Then $\pi_0(G, 1)$ may be turned into a group, and, in view of 2.1, the homotopy groups of G may be defined,[4] and we have

2.2. $\pi_n(G, 1) = H_n(\bar{G})$ *for each integer* $n \geq 0$.

3. *Paths and Loops.*—Let K be a c.s.s. complex, K_n the set of its n-simplices. Let $\varphi \in K_0$ be a fixed 0-simplex, the *base point* of K, and let $\varphi^n = \varphi\eta^0 \ldots \eta^{n-1}$. If $\tau \in K_{n+1}$, then we denote by $\tau\epsilon_{n+1}$ its $(n+1)$th vertex $\tau\epsilon^n \ldots \epsilon^0$. Let $\sigma \in K_n$. An *n-path of K ending at σ* is a sequence $(\sigma_1, \ldots, \sigma_{2l})$ of $(n+1)$-simplices of K such that

$$\sigma_1\epsilon^{n+1} = \varphi^n, \qquad \sigma_{2j-1}\epsilon_{n+1} = \sigma_{2j}\epsilon_{n+1}, \qquad \text{for} \qquad 1 \leq j \leq l;$$

$$\sigma_{2l}\epsilon^{n+1} = \sigma, \qquad \sigma_{2j}\epsilon^{n+1} = \sigma_{2j+1}\epsilon^{n+1}, \qquad \text{for} \qquad 1 \leq j < l.$$

It is called a *reduced n-path* if in addition

$$\sigma_{2j+1} \neq \sigma_{2j}, \qquad \sigma_{2j} \neq \sigma_{2j+1}, \qquad \text{for all } j$$

A (reduced) n-path ending at φ^n is called a (*reduced*) *n-loop.*

A *tree* of K is a connected subcomplex $T \subset K$ which contains no reduced loops.

A *maximal tree* $T \subset K$ is a tree with $T_0 = K_0$. Roughly speaking, a (maximal) tree is a connected 1-dimensional subcomplex containing no closed loops (but all vertices).

4. *The Homotopy Groups.*—Let K be a connected c.s.s. complex with base point φ. We now define a c.s.s. group G as follows. Choose a maximal tree $T \subset K$. Let G_n be the free (non-Abelian) group with a generator $\bar{\sigma}$ for each $\sigma \in K_{n+1}$ and a relation $\bar{\tau} = 1$ whenever $\tau \in T_{n+1}$ or $\tau = \rho\eta^n$ for some $\rho \in K_n$, and define the (right) homomorphisms $\epsilon^i: G_n \to G_{n-1}$ and $\eta^i: G_{n-1} \to G_n$ by

$$\bar{\sigma}\epsilon^i = \overline{\sigma\epsilon^i}, \qquad \bar{\sigma}\eta^i = \overline{\sigma\eta^i}, \qquad \text{for } 0 \leq i < n;$$

$$\bar{\sigma}\epsilon^n = \overline{\sigma\epsilon^n}(\overline{\sigma\epsilon^{n+1}})^{-1}, \qquad \bar{\sigma}\eta^n = \overline{\sigma\eta^n}.$$

It is easily verified that G so defined is a c.s.s. group.

As in Note III, let $jK: K \to S|K|$ be the natural monomorphism of K into the simplicial singular complex of its geometrical realization. Let $\varphi_s = (jK)\varphi$. Then our main result is

4.1. *For each integer $n > 0$ there exists an isomorphism $g_*: \pi_n(S|K|, \varphi_s) \approx H_{n-1}(\tilde{G})$. This isomorphism is natural on the category of c.s.s. complexes with base point.*

Thus the homotopy groups of $|K|$ may be expressed in terms of the free groups G_n and the homomorphisms $\epsilon^i: G_n \to G_{n-1}$. If K is finite in every dimension (in particular, if K is a simplicial complex), then G_n is *finitely generated* for all n.

5. *The Homology Groups.*—Define a c.s.s. group A as follows. Let $A_n = G_n/[G_n, G_n]$, and let the homomorphisms ϵ^i and η^i be those induced by the corresponding homomorphisms of G. Thus A is "*G made abelian.*" Denote by $k: G \to A$ the natural epimorphism and by $\tilde{k}: \tilde{G} \to \tilde{A}$ the induced chain map. Let $h_*: \pi_n(S|K|, \varphi_s) \to H_n(S|K|)$ be the Hurewicz homomorphism. Then we have, for each integer $n > 0$:

5.1 *There exists a natural isomorphism $a_*: H_n(S|K|) \approx H_{n-1}(\tilde{A})$.*

5.2 *Commutativity holds in the diagram*

$$\begin{array}{ccc} \pi_n(S|K|, \varphi_s) & \xrightarrow{g_*} & H_{n-1}(\tilde{G}) \\ \downarrow{\scriptstyle h_*} & & \downarrow{\scriptstyle \tilde{k}_*} \\ H_n(S|K|) & \xrightarrow{a_*} & H_{n-1}(\tilde{A}) \end{array}$$

6. *The Maximal Tree T.*—We define a c.s.s. complex B as follows. An n-simplex of B is a pair (γ, σ), where $\gamma \in G_n$ and $\sigma \in K_n$. Its faces and degeneracies are given by

$$(\gamma, \sigma)\epsilon^i = (\gamma\epsilon^i, \sigma\epsilon^i), \qquad (\gamma, \sigma)\eta^i = (\gamma\eta^i\ \sigma\eta^i), \qquad \text{for } 0 \leq i < n;$$

$$(\gamma, \sigma)\epsilon^n = (\gamma\epsilon^n\bar{\sigma}, \sigma\epsilon^n), \qquad (\gamma, \sigma)\eta^n = (\gamma\eta^n, \sigma\eta^n).$$

Then the c.s.s. map $p: B \to K$ defined by $p(\gamma, \sigma) = \sigma$ is a fiber map. We identify G with the fiber of p over φ by setting $\gamma = (\gamma, \varphi^n)$ for $\gamma \in G_n$.

For each reduced n-path $b = (\sigma_1, \ldots, \sigma_{2l})$ of K ending at σ, define $t(b) \in B_n$ by

$$t(b) = (\gamma, \sigma), \qquad \text{where } \gamma = \prod_{j=1}^{l} (\bar{\sigma}_{2j-1}{}^{-1}\bar{\sigma}_{2j}).$$

6.1. *The function t defines a one-to-one correspondence between the reduced n-paths (resp. reduced n-loops) of K and the n-simplices of B (resp. G).*

If we identify the simplices of B with the reduced paths of K by this correspondence t, i.e., if we consider B as a complex with the reduced n-paths of K as its n-simplices and G as a c.s.s. group with the reduced n-loops of K as its n-simplices, then

6.2. *The fiber map $p: B \to K$ with fibre G over φ is independent of the choice of the maximal tree T. Changing the maximal tree merely means changing the bases of the free groups G_n.*

7. The *Isomorphism* g_*.—Application of the functor[5] Ex^∞ to the fiber map $p: B \to K$ yields a fiber map $Ex^\infty p: Ex^\infty B \to Ex^\infty K$ with fiber $Ex^\infty G$ over $\psi = e^\infty{}_k\varphi$. Clearly $Ex^\infty G$ is also a c.s.s. group. It may be shown that $Ex^\infty B$ is contractible, i.e., $\pi_n(Ex^\infty B) = 0$ for all n. The exactness of the homotopy sequence of a fiber map then implies that $\partial_*: \pi_n(Ex^\infty K, \psi) \backsimeq \pi_{n-1}(Ex^\infty G, 1)$ for all n.

Let $\psi_s = (jEx^\infty K)\psi$. Then the isomorphism $g_*: \pi_n(S|K|, \varphi_s) \backsimeq H_{n-1}(\bar{G})$ is defined as the composition of the isomorphisms in the following diagram.

$$\begin{array}{ccccc} \pi_{n-1}(Ex^\infty G, 1) & \xleftarrow{\partial_*} & \pi_n(Ex^\infty K, \psi) & \xrightarrow{jEx^\infty K_*} & \pi_n(S|Ex^\infty K|, \psi_s) \\ \uparrow{\scriptstyle e^\infty{}_{G*}} & & & & \uparrow{\scriptstyle S|e^\infty{}_{K*}|} \\ H_{n-1}(\bar{G}) = \pi_{n-1}(G, 1) & & & & \pi_n(S|K|, \varphi_s) \end{array}$$

8. *The Groups* $\bar{G}_n$.—Because $\bar{G}_n \subset G_n$ and G_n is free, it follows that $\bar{G}_n$ is free. A (in general infinite) set of generators of $\bar{G}_n$ can be found as follows. For each integer i with $0 \leq i \leq n$, define a subgroup $G_n{}^i \subset G_n$ by

$$G_n{}^i = G_n \cap \textit{kernel } \epsilon^0 \cap \ldots \cap \textit{kernel } \epsilon^{i-1}.$$

It is easily verified that the function $\epsilon^i\eta^i$ is a *coset function* of $G_n{}^{i+1}$ in $G_n{}^i$, i.e., that, for $\sigma, \tau \in G_n{}^i$, $\sigma\tau^{-1} \in G_n{}^{i+1}$ implies $\sigma\epsilon^i\eta^i = \tau\epsilon^i\eta^i$ and conversely. As a set of generators of $G_n{}^0 = G_n$ is given, now iterated application of the Schreier theorem[6] yields a set of generators for $\bar{G}_n = G_n{}^n$.

Thus the problem of computing the homotopy groups of a finite simplicial complex is brought back to the group theoretical problem of computing the homology groups of a (non-Abelian) chain complex with a countably generated free group in every dimension.

[1] Cf. S. Eilenberg and J. A. Zilber, *Ann. Math.*, **51**, 499–513, 1950.

[2] D. M. Kan, these PROCEEDINGS, **42**, 419, 1956. This will be referred to as "Note III."

[3] The results of this section are due to J. C. Moore.

[4] Analogous to the definition of the homotopy groups of a cubical complex which satisfies the extension axiom; see D. M. Kan, these PROCEEDINGS, **41**, 1092–1096, 1955.

[5] In this section we freely use the notation and results of Note III.

[6] Cf. A.G. Kurosch, *Gruppentheorie* (Berlin, 1953).

FOUNDATIONS OF QUASIGROUPS

By Sherman K. Stein

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A quasigroup is a multiplicative system $xy = z$ satisfying three conditions: (i) x and y determine z, (ii) x and z determine y, (iii) y and z determine x. The most thoroughly studied quasigroups satisfy the condition of associativity

$$ab \cdot c = a \cdot bc$$

and are called groups.

As observed by E. L. Post and others, conditions i, ii, and iii are symmetric; the operations of "solving" and "evaluating" play similar roles. Therefore quasigroups come in clusters of six. One could think of the six multiplications $xy = z$, $yx = z$, $xz = y$, $zx = y$, $yz = x$, $zy = x$ as conjugates of the original quasigroup $xy = z$ and representing different points of view rather than different systems. However, if the original quasigroup Q satisfies an identity ν, then the conjugates of Q may satisfy quite different identities which could be termed conjugates of ν (see Theorem 1). If ν is equal to its conjugates it is termed invariant.

The operation of conjugation provides a method and point of view for the study of quasigroups and identities which illuminates much of the work on the axiomatization of groups by A. Tarski, M. Ward, and H. Furstenberg, for example, and suggests that the study of conditions other than associativity may be very fruitful. The following theorems illustrate this point of view.

Theorem 1. *The identity $ab \cdot ac = bc$ is a conjugate of associativity.*

The proof of this theorem is included because it is short and illustrates well the relation between conjugate quasigroups. The associative identity $a \cdot bc = ab \cdot c$ can be written: $bc = x$, $ax = y$, $ab = z$ imply $zc = y$. In the conjugate quasigroup obtained by interchanging the second and third letters, this is equivalent to: $bx = c$, $ay = x$, $az = b$ imply $zy = c$, or simply $az \cdot ay = zy$, which is equivalent to $ab \cdot ac = bc$.

Thus the theory of groups is equivalent to the study of systems satisfying the identity $ab \cdot ac = bc$. This fact suggests that systems satisfying other identities (even those usually in opposition to associativity) may merit study.

Theorem 2. *A quasigroup satisfies the identity $ab \cdot bc = ac$ if and only if it is a group which is a power of the group of order 2 or, equivalently, if all its conjugates are groups.*

In general, however, of all its conjugates, only the transpose of a group is again a group.

Theorem 3. *There exists no finite quasigroup of order $4k + 2$ satisfying the identity $a \cdot bc = ab \cdot ac$. For all other orders there do exist such quasigroups.*

The identity $a \cdot bc = ab \cdot ac$ or "left distributivity" has been studied by C. Burstin and W. Mayer, M. Knaster, and T. Mituhisa.

Theorem 4. *A quasigroup of order $4k + 2$ containing an element which is equal to its square cannot have a transitive group of automorphisms.*

Many quasigroups are homogeneous in the sense that their group of automorphisms is transitive. The only homogeneous group is of order 1. This illustrates

a second symmetry which quasigroups can possess but which groups (with more than one element) lack.

Definition: A quasigroup is medial if it satisfies the identity $ab \cdot cd = ac \cdot bd$. (Medial quasigroups, under different names, have been considered by I. M. H. Etherington, D. C. Murdoch, R. H. Bruck, M. Knaster, K. Toyoda, and T. Mituhisa.)

THEOREM 5. *Each of the following conditions on Q is equivalent to the condition that Q be medial.*

1. *For any algebra A and homomorphisms f, g: $A \to Q$ the product fg: $A \to Q$ is a homomorphism.*
2. *Q satisfies an invariant identity of the form $ab \cdot cd = (t(a) \cdot t(b))\ (t(c) \cdot t(d))$, where t is a nonidentical permutation of a, b, c, d.*
3. *Q satisfies (i) $xa = by$, $za = bw$ imply $xw = zy$, and (ii) the simultaneous equations $ad \cdot x = y \cdot dc$, $ab \cdot x = y \cdot bc$ have a solution x, y.*
4. *Q satisfies: $ab = a'b'$, $cd = c'd'$ imply $ac \cdot bd = a'c' \cdot b'd'$.*
5. *Q satisfies: $pq = rs$ implies $xp \cdot yq = xr \cdot ys$.*

THEOREM 6. *A smooth convex planar curve K is a conic if and only if the following quasigroup is medial. Let T be a fixed tangent to K with point of contact A. If a, b are in $K - A$, let ab be the point of contact of the tangent to K passing through the intersection of T with the line through a and b (define $aa = a$).*

Proofs of these theorems, along with a general development of the theory and implications of conjugation, will appear elsewhere.

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*SOME MOLECULAR COLLISION INTEGRALS FOR POINT ATTRACTION AND REPULSION POTENTIALS**

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If the pairs of molecules in a dilute gas interact with an intermolecular energy of the form $\Phi = d/r^{\delta}$, the collision integrals which determine the transport properties can be written[1]

$$\Omega^{(l,s)} = \left(\frac{\pi kT}{2\mu}\right)^{1/2}\left(\frac{\delta}{kT}\right)^{2/\delta} (d^2)^{1/\delta}\, \Gamma(s + 2 - (2/\delta))A^{(l)}(\delta). \qquad (1)$$

Here μ is the reduced mass of the colliding molecules. The following constants are evaluated:

Repulsive interaction potential, d positive:

$$A^{(1)}(2) = 0.397601, \quad A^{(2)}(2) = 0.527843,$$
$$A^{(1)}(3) = 0.3115, \quad A^{(2)}(3) = 0.3533;$$

Attractive interaction potential, d negative:

$$A^{(1)}(2) = 0.806907, \quad A^{(2)}(2) = 0.710970,$$
$$A^{(1)}(3) = 0.6750, \quad A^{(2)}(3) = 0.4641,$$
$$A^{(1)}(6) = 0.434, \quad A^{(2)}(6) = 0.328,$$
$$A^{(3)}(6) = 0.502, \quad A^{(4)}(6) = 0.420.$$

Although the $A^{(l)}(\delta)$ have been previously tabulated and used for repulsive potentials with $\delta \geq 4$, the notion of the $A^{(l)}(\delta)$ for attractive potentials is new. It is somewhat surprising that these collision integrals should be finite. For large impact parameters the trajectory is deflected toward the center of force. As the impact parameter is decreased, the molecules tend to spiral about each other more and more, until, for a critical value of the impact parameter, true orbiting occurs. For an impact parameter slightly smaller than the critical value, the molecules spiral a few times about each other as they approach each other with a relative velocity which becomes progressively greater. Since our hypothetical molecules have no rigid cores, the two molecules pass through each other ($r = 0$) with infinite velocity and then separate, the outward leg of the trajectory being geometrically similar to the inward path. The calculations of the collision integrals for these attractive potentials had to be carried out numerically.

The second virial coefficients for either $\delta = 2$ or $\delta = 3$ would be infinite,[2] but the transport coefficients are finite. The constants for $\delta = 2$ were readily evaluated in closed form. For the repulsive potential with $\delta = 3$ an expansion developed by Mott-Smith[3] was used. For the attractive potential with $\delta = 3$ the $A^{(l)}(3)$ were evaluated numerically making use of elliptic integrals (of the first kind) for the angles of deflection. For $\delta = 6$ the $A^{(l)}(6)$ were taken directly from the $Z^{(l,\,s)}$ integrals which Mason[4] tabulated for the Buckingham (6-exp) potential in the limit of low temperature where the exponential repulsive part of the potential does not affect the transport integrals.

The collision integrals are defined in the following way.[5] The collision of two molecules is considered as the equivalent one-body problem. A particle with reduced mass $\mu = m_1m_2/(m_1 + m_2)$ approaches a fixed center of force with initial speed g and impact parameter b. The potential energy of interaction is given by $\Phi = d/r^\delta$, where d is a constant and r the distance of the incoming particle from the scattering center. Figure 1 shows the trajectory. The particle is deflected through an angle of deflection χ, and the distance of closest approach is r_m.

Now, if we define

$$y = \frac{b}{r}, \qquad y_m = \frac{b}{r_m}, \qquad y_0 = \left[\frac{{}^1/_2\mu g^2}{\delta d}\right]^{1/\delta}, \tag{2}$$

the angle of deflection may be written as a function of y_0:

$$\chi(y_0) = \pi - 2\theta_m = \pi - 2\int_0^{y_m(y_0)} \left[1 - y^2 - \frac{1}{\delta}\left(\frac{y}{y_0}\right)^\delta\right]^{-1/2} dy. \tag{3}$$

The upper limit on the integral is the first positive root of the polynomial in the denominator of the integrand.

Then the collision cross-sections for the various transport properties, in the case of repulsive potentials, are given by[6]

$$Q^{(l)} = 2\pi\left[\frac{\delta d}{{}^1/_2\mu g^2}\right]^{2/\delta} A^{(l)}(\delta), \tag{4}$$

where the molecular collision integral, $A^{(l)}(\delta)$ is given by

$$A^{(l)}(\delta) = \int_0^\infty (1 - \cos^l \chi) y_0 dy_0. \tag{5}$$

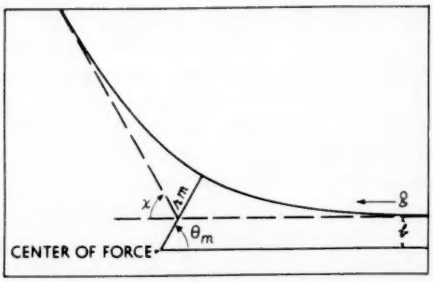

FIG. 1.—A typical trajectory for the equivalent one-body problem with a repulsive potential.

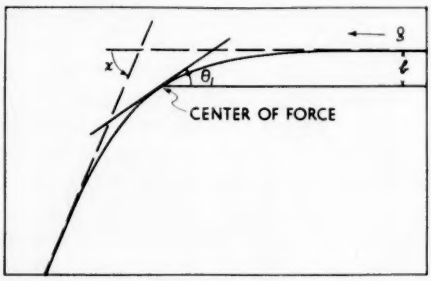

FIG. 2.—A typical trajectory for the equivalent one-body problem with an attractive potential, in the case where $\beta < \beta_{crit}$ and the particle passes through the center of force.

When the potential is attractive, $d = -a$ and the potential has the form

$$\Phi(r) = -ar^{-\delta}. \tag{6}$$

Also, let us define

$$\beta = b\left[\frac{1/2\mu g^2}{a\delta}\right]^{1/\delta}. \tag{7}$$

It is easily seen that with β, which is a real, positive quantity, equation (3) becomes, for an attractive potential,

$$\chi(\beta) = \pi - 2\theta_2 = \pi - 2\int_0^{y_m(\beta)}\left[1 - y^2 + \frac{1}{\delta}\left(\frac{y}{\beta}\right)^{\delta}\right]^{-1/2} dy. \tag{8}$$

If β is greater than β_{crit}, the polynomial in the denominator of the integrand has a first positive root, $y_m(\beta)$. However, if β is less than β_{crit}, $y_m(\beta) = \infty$, corresponding to the fact that the incoming particle actually passes through the center of force, $r_m = 0$, and then goes out again. In this case the angle of deflection is given by

$$\chi(\beta) = -2\theta_1 = -2\int_0^{\infty}\left[1 - y^2 + \frac{1}{\delta}\left(\frac{y}{\beta}\right)^{\delta}\right]^{-1/2} dy \tag{9}$$

rather than by equation (8). Such a trajectory is shown in Figure 2.

For the attractive potentials the various collision cross-sections are given by[6]

$$Q^{(l)} = 2\pi\left(\frac{\delta a}{1/2\mu g^2}\right)^{2/\delta} A^{(l)}(\delta), \tag{10}$$

where

$$A^{(l)}(\delta) = \int_0^{\infty}(1 - \cos^l\chi)\beta\, d\beta. \tag{11}$$

I. CENTERS OF REPULSION

For repulsive potentials, when δ is 2 or greater, the $A^{(l)}(\delta)$ are finite. The case of $\delta = 1$, however, is a potential of sufficiently long range that the integral for $A^{(l)}(1)$ is infinite. In this case, the angle of deflection is

$$\chi(y_0) = 2 \sin^{-1} [(1 + 4y_0^2)^{-1/2}], \tag{12}$$

and the integral in equation (5) diverges.

a) $\delta = 2$; Repulsive Potential.—The repulsive, inverse-square potential is of interest because the integrations may be carried out analytically.[7] First of all, the angle of the deflection from equation (3) is

$$\chi(y_0) = \pi - 2 \int_0^{y_m} \left[1 - \left(1 + \frac{1}{2y_0^2}\right) y^2\right]^{-1/2} dy. \tag{13}$$

Let $x = 2y_0^2$; then $y_m = (1 + 1/x)^{-1/2}$;

$$\chi(x) = \pi - 2\left(1 + \frac{1}{x}\right)^{-1/2} \sin^{-1}\left[y\left(1 + \frac{1}{x}\right)^{1/2}\right]\Bigg|_0^{\left(1+\frac{1}{x}\right)^{-1/2}},$$

$$\chi(x) = \pi\left[1 - \left(1 + \frac{1}{x}\right)^{-1/2}\right]. \tag{14}$$

With this change of variables, equation (5) becomes

$$A^{(l)}(2) = {}^1/_4 \int_0^\infty (1 - \cos^l \chi)\, dx. \tag{15}$$

Now let $x = z^2/(1 - z^2)$; then $\chi = \pi(1 - z)$. Substituting in equation (15) and integrating by parts,

$$A^{(1)}(2) = -{}^1/_2 + \frac{\pi}{4} \int_0^1 \frac{\sin \pi z}{1 - z^2}\, dz, \tag{16}$$

$$A^{(2)}(2) = -\frac{\pi}{4} \int_0^1 \frac{\sin 2\pi z}{1 - z^2}\, dz. \tag{17}$$

But it is easy to show that

$$\int_0^1 \frac{\sin n\pi z}{1 - z^2}\, dz = {}^1/_2(-1)^n\, [Si(2n\pi) - 2Si(n\pi)], \tag{18}$$

where[8]

$$Si(x) = \int_0^x \frac{\sin z}{z}\, dz. \tag{19}$$

Thus we obtain

$$A^{(1)}(2) = 0.397601, \tag{20}$$

$$A^{(2)}(2) = 0.527843. \tag{21}$$

b) $\delta = 3$; Repulsive Potential.—In the case of the inverse-cube repulsive potential, we were able to make use of a method which has been developed by Mott-Smith[3] to evaluate the $A^{(l)}(\delta)$. Through the application of Bürmann's theorem[9] he has found that equation (5) can be written as

$$A^{(l)}(\delta) = 2^{-2/\delta} \int_0^\pi (1 - \cos^l \chi)\, F(\chi) \sin \chi\, d\chi, \tag{22}$$

where the $F(\chi) \sin \chi$ is given by

$$F(\chi) \sin \chi = \cos \frac{\chi}{2} \sum_{\rho=0}^{\infty} D_\rho \left(\sin \frac{\chi}{2} \right)^{\rho - 1 - 2/\delta}. \tag{23}$$

The D_ρ are constants, depending only on δ.

$$D_\rho = {}^1/_4 \left(\frac{2}{\delta} \right)^{1+2/\delta} \frac{1}{\rho!} \left\{ \frac{d^\rho}{dz^\rho} (1+z)^{2/\delta - 2} \left(1 + \frac{\delta}{2} z \right) \left(\frac{z}{\sin {}^1/_2 H(z)} \right)^{\rho - 2/\delta} \right\}_{z=0}, \tag{24}$$

with the $H(z)$ given by

$$H(z) = - \sum_{s=1}^{\infty} \binom{-{}^1/_2}{s} z^s \sum_{t=0}^{s} (-1)^t \binom{s}{t} \frac{\Gamma({}^1/_2 \delta t + {}^1/_2) \Gamma(-s + {}^1/_2)}{\Gamma({}^1/_2 \delta t - s + 1)}. \tag{25}$$

For $\delta = 3$ we obtain the results given in Table 1.

TABLE 1

THE MOTT-SMITH COEFFICIENTS D_ρ FOR $\delta = 3$

ρ	D_ρ	ρ	D_ρ
0	0.25438	3	−0.00282
1	0.12487	4	0.00317
2	0.01019		

Substitution of equation (23) into equation (22) and integration give the general expression

$$A^{(l)}(\delta) = -2^{-2/\delta} \sum_{\rho=0}^{\infty} D_\rho \sum_{n=1}^{l} (-1)^n 2^{n+1} \binom{l}{n} \left(\frac{\delta}{\delta\rho + 2\delta n - 2} \right). \tag{26}$$

The two cases which are of interest are then

$$A^{(1)}(3) = 2^{4/3} \sum_{\rho=0}^{\infty} D_\rho \left[\frac{1}{\rho + {}^4/_3} \right], \tag{27}$$

$$A^{(2)}(3) = 2^{7/3} \sum_{\rho=0}^{\infty} D_\rho \left[\frac{1}{\rho + {}^4/_3} - \frac{1}{\rho + {}^{10}/_3} \right]. \tag{28}$$

Thus we obtain

$$A^{(1)}(3) = 0.3115, \tag{29}$$

$$A^{(2)}(3) = 0.3533. \tag{30}$$

II. CENTERS OF ATTRACTION

When the potential is attractive, there is a singularity in the expression for the angle of deflection. The Mott-Smith formulation is no longer applicable, so we proceed as follows.

There exists a critical value of $b = b_{\text{crit}}$ for any relative kinetic energy ${}^1/_2 \mu g^2$ such that for b less than b_{crit} there are no positive roots to the equation

$$f(y) = 1 - y^2 + cy^\delta = 0, \tag{31}$$

where

$$c = \frac{1}{\delta \beta^\delta} = \frac{a}{({}^1/_2 \mu g^2) b^\delta}, \tag{32}$$

and for $b = b_{crit}$, $c = c_{crit}$. This critical value may be obtained by noting that equation (31) has a double root at the critical value. Therefore, both $f(y)$ and $f'(y)$ vanish for the same positive root, y_{crit}.

$$f'(y) = y^{-1}[-2y^2 + c\delta y^\delta] = 0. \tag{33}$$

One may combine equations (31) and (33), to solve for this root

$$y_{crit} = \left[\frac{\delta}{\delta - 2}\right]^{1/2}, \tag{34}$$

and then go back and obtain the critical value of c,

$$c_{crit} = 2\delta^{-\delta/2}[\delta - 2]^{\delta/2-1}. \tag{35}$$

Thus

$$\beta_{crit} = 2^{-1/\delta}\left(\frac{\delta}{\delta - 2}\right)^{(1/2)-(1/\delta)}. \tag{36}$$

a) $\delta = 2$; Attractive Potential.—For the attractive case, with $\delta = 2$, the angle of deflection is, from equations (8) and (9),

$$\chi(\beta) = \pi - 2\int_0^{[1-1/2\beta^2]^{-1/2}} \left[1 - \left(1 - \frac{1}{2\beta^2}\right)y^2\right]^{-1/2} dy, \quad \beta^2 > 1/2, \tag{37}$$

and

$$\chi(\beta) = -2\int_0^{\infty} \left[1 - \left(1 - \frac{1}{2\beta^2}\right)y^2\right]^{-1/2} dy, \quad \beta^2 < 1/2. \tag{38}$$

Thus

$$\chi(\beta) = \pi\left[1 - \left(1 - \frac{1}{2\beta^2}\right)^{-1/2}\right], \quad \beta^2 > 1/2; \tag{39}$$

$$\chi(\beta) = -2\left(\frac{1}{2\beta^2} - 1\right)^{-1/2} \ln\left\{\left(\frac{1}{2\beta^2} - 1\right)^{1/2} y + \left[1 + \left(\frac{1}{2\beta^2} - 1\right)y^2\right]^{1/2}\right\}\Bigg|_0^{\infty}$$

$$= -\infty, \quad \beta^2 < 1/2. \tag{40}$$

In spite of the infinite value of the angle of deflection for all values of β^2 less than $1/2$, one may still obtain the collision integrals. Equation (11) is split into two parts:

$$A^{(l)}(2) = \int_0^{2^{-1/2}} (1 - \cos^l \chi)\beta\, d\beta + \int_{2^{-1/2}}^{\infty} (1 - \cos^l \chi)\beta\, d\beta$$

$$= G^{(l)} + H^{(l)}. \tag{41}$$

The second of these, the $H^{(l)}$, are obtained in closed form. Taking the expression for χ from equation (39), and letting $x = [1 - (2\beta^2)^{-1}]$, it is found that

$$H^{(1)} = \frac{1}{2}\int_0^{\infty} \cos^2\frac{\pi}{2}x\, d\left(\frac{1}{x^2 - 1}\right), \tag{42}$$

$$H^{(2)} = \frac{1}{4}\int_0^{\infty} \sin^2 \pi x\, d\left(\frac{1}{x^2 - 1}\right). \tag{43}$$

These expressions may be integrated by parts to give

$$H^{(1)} = \frac{\pi}{4}\int_1^\infty \frac{\sin \pi x}{1 - x^2}\,dx, \tag{44}$$

$$H^{(2)} = -\frac{\pi}{4}\int_1^\infty \frac{\sin 2\pi x}{1 - x^2}\,dx. \tag{45}$$

These may be rewritten as

$$H^{(1)} = \frac{\pi}{4}\left\{\int_0^\infty \frac{\sin \pi x}{1 - x^2}\,dx - \int_0^1 \frac{\sin \pi x}{1 - x^2}\,dx\right\}, \tag{46}$$

$$H^{(2)} = \frac{\pi}{4}\left\{-\int_0^\infty \frac{\sin 2\pi x}{1 - x^2}\,dx + \int_0^1 \frac{\sin 2\pi x}{1 - x^2}\,dx\right\}. \tag{47}$$

The first integral in each case is found in Bierens de Haan,[10] and the second is given by equation (18). Thus

$$H^{(1)} = \frac{\pi}{4}\left\{[Ci(\pi)\sin \pi - Si(\pi)\cos \pi] + {}^1/_2\, Si(2\pi) - Si(\pi)\right\}, \tag{48}$$

$$H^{(2)} = \frac{\pi}{4}\left\{-[Ci(2\pi)\sin 2\pi - Si(2\pi)\cos 2\pi] + {}^1/_2 Si(4\pi) - Si(2\pi)\right\}. \tag{49}$$

Therefore,

$$H^{(1)} = \frac{\pi}{8}\,Si(2\pi) = 0.556907, \tag{50}$$

$$H^{(2)} = \frac{\pi}{8}\,Si(4\pi) = 0.585970. \tag{51}$$

The values of the integrals $G^{(i)}$ may be approximated in the following way.

$$G^{(i)} = {}^1/_2 \int_0^{1/2} (1 - \cos^i \chi)\,d\beta^2. \tag{52}$$

If the upper limit on the integral for the angle of deflection is taken to be an arbitrarily large, but finite number z,

$$\chi = -2\left(\frac{1}{2\beta^2} - 1\right)^{-1/2} \ln\left\{\left(\frac{1}{2\beta^2} - 1\right)^{1/2} z + \left[1 + \left(\frac{1}{2\beta^2} - 1\right) z^2\right]^{1/2}\right\}. \tag{53}$$

With this expression for the angle of deflection, the integrand of equation (52) is a very rapidly oscillating function of β^2, oscillating between 0 and 2 for $G^{(1)}$ and between 0 and 1 for $G^{(2)}$. As z becomes very large, the peaks become very close, and the average value of the integrand becomes very close to 1 for the $G^{(1)}$ and to $^1/_2$ for the $G^{(2)}$. Thus, in the limit that z becomes infinite, corresponding to the actual kinetic theory problem,

$$G^{(1)} = 0.25, \tag{54}$$

$$G^{(2)} = 0.125. \tag{55}$$

Thus we obtain

$$A^{(1)}(2) = G^{(1)} + H^{(1)} = 0.806907, \tag{56}$$

$$A^{(2)}(2) = G^{(2)} + H^{(2)} = 0.710970. \tag{57}$$

b) $\delta = 3$; Attractive Potential.—The calculations of the collision integrals for the inverse-cube attractive potential are somewhat more difficult.[11] Figure 3 shows

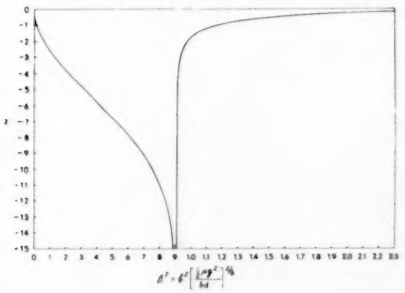

Fig. 3.—Angle of deflection χ versus β^2 for attractive potential with $\delta = 3$.

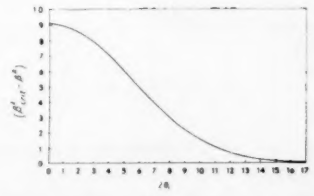

Fig. 4.—$(\beta^2_{crit} - \beta^2)$ versus $2\theta_1$ when $\beta \leq \beta_{crit}$.

the angle of deflection χ as a function of β^2. As in the previous case, there is a singular point. For $\delta = 3$, $\beta_{crit} = (3/4)^{1/6} = 0.95318$ and $y_m(\beta)$ varies from unity for $\beta = \infty$ to $y_m(\beta_{crit}) = 3^{1/2}$. Because of the singular point it is convenient to divide the integration of $A^{(l)}(3)$ into two parts:

$$A^{(l)}(3) = C_1^{(l)}(3) + C_2^{(l)}(3), \tag{58}$$

where

$$C_1^{(l)}(3) = \tfrac{1}{2}\int_0^{(3/4)^{1/2}} [1 - \cos^l(2\theta_1)]\, d\beta^2, \tag{59}$$

$$C_2^{(l)}(3) = \tfrac{1}{2}\int_{(3/4)^{1/2}}^{\infty} [1 - (-1)^l \cos^l(2\theta_2)]\, d\beta^2. \tag{60}$$

Here θ_1 and θ_2 are given in equations (9) and (8), respectively. The variation of $\beta^2_{crit} - \beta^2$ as a function of $2\theta_1$ is shown in Figure 4.

In evaluating $C_1^{(l)}(3)$, it is convenient to change the variable of integration[12] from β^2 to q^2, where q is minus the (only) real root of the polynomial in the denominator of equation (9):

$$\beta^2 = q^2[3(1 - q^2)]^{-3/2}. \tag{61}$$

Since the real root is negative in the range $0 < \beta^2 < (3/4)^{1/3}$, q is positive. Making the change of variables,

$$\theta_1 = \left(\frac{q^3}{1 - q^2}\right)^{1/2} \int_0^{\infty} \left[\frac{q^3}{1 - q^2} - \frac{q^3}{1 - q^2}\, y^2 + y^3\right]^{-1/2} dy, \tag{62}$$

which is an incomplete elliptic integral of the first kind.[13] Thus

$$\theta_1 = \left(\frac{q^3}{1 - q^2}\right)^{1/2} gF(\varphi, k), \tag{63}$$

where

$$g = \left[\frac{1 - q^2}{q^2(3 - q^2)}\right]^{1/4}, \tag{64}$$

$$k^2 = {}^1/_2 + {}^1/_4(3 - 2q^2)\,[(1 - q^2)\,(3 - q^2)]^{-1/2}, \tag{65}$$

$$\varphi = \cos^{-1}\,[q^2 - 2 + \{(1 - q^2)\,(3 - q^2)\}^{1/2}]. \tag{66}$$

Since

$$\frac{d(\beta^2)}{d(q^2)} = 3^{-5/4}\,\frac{(3 - q^2)}{(1 - q^2)^{5/4}},$$

the first integral, $C_1^{(l)}(3)$, is now given by

$$C_1^{(l)}(3) = \frac{1}{2 \cdot 3^{5/4}} \int_0^{1/4} [1 - \cos^l(2\theta_1)]\,\frac{(3 - q^2)}{(1 - q^2)^{5/4}}\,d(q^2). \tag{67}$$

Figures 5 and 6 show the integrands for $l = 1$ and for $l = 2$, respectively, as functions of q^2.

It is seen that the integrands oscillate wildly as q^2 approaches 0.75. The integrals are subdivided into four ranges of q^2. For the smaller values of q^2 numerical integration is satisfactory, making use of the elliptic functions. However, for the larger values of q^2 it is convenient to use $2\theta_1$ as the integration variable and develop approximate expressions for $d(\beta^2)/d(2\theta_1)$ which will allow analytic evaluation of the required integrals. Thus $C_1^{(l)}(3)$ is divided into the following four ranges: (1) $0 \leq q^2 \leq 0.52$, (2) $0.52 \leq q^2 \leq 0.68$, (3) $0.68 \leq q^2 \leq 0.748$, and (4) $0.748 \leq q^2 \leq 0.75$. In the first range, the integrands were evaluated for $q^2 = 0$, 0.04, 0.08, 0.12, . . . , 0.40, 0.42, 0.44, . . . , 0.52, using four-point Lagrangian interpolations together with the elliptic integral tables of Byrd and Friedman.[14] The contributions to $C_1^{(1)}(3)$ and $C_1^{(2)}(3)$ from this range were found to be 0.2449 and 0.1136, respectively. In the second region, an expression for $d\beta^2/d(2\theta_1)$ is obtained by differentiating the empirical equation

$$\beta_{crit}^2 - \beta^2 = 1.062129 - 0.0640403(2\theta_1) - 0.0092255(2\theta_1)^2 + 0.0006563(2\theta_1)^3. \tag{68}$$

The constants in this cubic equation were obtained by requiring that the values of β^2 for $q^2 = 0.52$, 0.58, 0.64, and 0.68 be the values calculated directly from the tables of elliptic integrals using four-point Lagrangian interpolation. Spot checks for different values of q^2 indicated that equation (68) gives values of $\beta_{crit}^2 - \beta^2$ accurate to within 0.05 per cent through the second range. The contributions to $C_1^{(1)}(3)$ and $C_1^{(2)}(3)$ from this region are 0.1020 and 0.0690, respectively. In the third range, an expression for $d\beta^2/d(2\theta_1)$ is obtained by differentiating the empirical equation

$$\beta_{crit}^2 - \beta^2 = (-30.419 + 7.5241(2\theta_1) - 0.21952(2\theta_1)^2 \exp(-\theta_1). \tag{69}$$

The functional form of equation (69) was suggested by the asymptotic behavior of β^2, and the constants were adjusted so as to agree with the direct calculations for $q^2 = 0.68$, 0.727, and 0.748. Spot checks for different values of q^2 indicate that

equation (69) is accurate to within 0.5 per cent within the third range. The contributions to $C_1^{(1)}(3)$ and $C_1^{(2)}(3)$ from the third range are, respectively, 0.1217

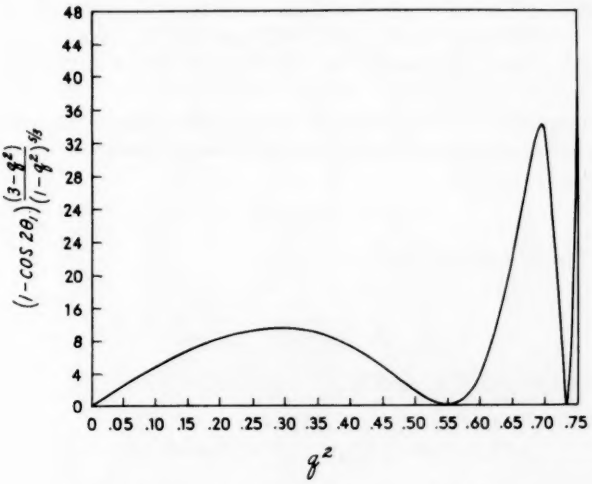

FIG. 5.—Integrand of $C_1^{(1)}$ (3) from equation (67).

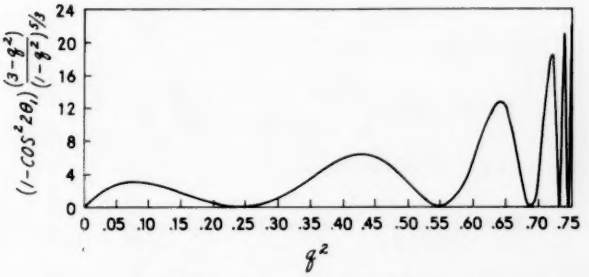

FIG. 6.—Integrand of $C_1^{(2)}$ (3) from equation (67).

and 0.0444. In the fourth region, use is made of the true asymptotic expansion of the elliptic function, $F(\varphi, k)$. It is convenient to express $F(\varphi, k)$ in the form[15]

$$F(\varphi, k) = 2\,K(k) - F(\pi - \varphi, k). \tag{70}$$

As $q^2 \to 0.75$, $k \to 1$ and $\varphi \to 2\pi/3$. It is easy to show that throughout the range $0.748 \leq q^2 \leq 0.75$, only a negligible error is made in replacing $F(\pi - \varphi, k)$ by its limiting value $F(\pi/3, 1) = \ln (2 - (3)^{1/2})$. When k is close to unity, $K(k)$ can be expanded in the form[16]

$$K(k) = \left(1 + \frac{t^2}{4}\right) \ln\left(\frac{4}{t}\right) - \frac{t^2}{4} + \ldots, \tag{71}$$

where $t = (1 - k^2)^{1/2}$. Thus, in the fourth range, we find that to within the required accuracy

$$\frac{d\beta^2}{d(2\theta_1)} = 36(48)^{1/3}(2 - 3^{1/2}) \exp(-\theta_1) = 17.528 \exp(-\theta_1). \tag{72}$$

Using equation (72), we carried out the integrations analytically to obtain the contributions 0.0026 and 0.0022 to $C_1^{(1)}(3)$ and $C_1^{(2)}(3)$, respectively, due to the fourth range. Thus, all together we find that $C_1^{(1)}(3) = 0.4712$ and $C_1^{(2)}(3) = 0.2292$.

To evaluate the $C_2^{(l)}(3)$, as defined in equation (60), it is convenient to change the variable of integration from β^2 to p^2, where p is the first positive root of the denominator in equation (8).

$$\beta^2 = p^2[3(p^2 - 1)]^{-2/3}. \tag{73}$$

Since $y_m(\beta) = p$, it follows that

$$\theta_2 = \left(\frac{p^3}{p^2 - 1}\right)^{1/2} \int_0^p \left[\frac{p^3}{p^2 - 1} - \frac{p^3}{p^2 - 1} y^2 + y^3\right]^{-1/2} dy, \tag{74}$$

which is another elliptic integral of the first kind.[17] Thus

$$\theta_2 = \left(\frac{p^3}{p^2 - 1}\right)^{1/2} g' F(\varphi', k'), \tag{75}$$

where

$$g' = 2[p(p^2 - 1)^{-1}(4p^2 - 3)^{1/2}]^{-1/2}, \tag{76}$$

$$k'^2 = 1/2 + 1/2(2p^2 - 3)(4p^2 - 3)^{-1/2}, \tag{77}$$

$$\varphi' = \sin^{-1}\left[\frac{3(4p^2 - 3)^{1/2} - 4p^2 + 3}{2(3 - p^2)}\right]^{1/2} \tag{78}$$

Equation (60) for the $C_2^{(l)}(3)$ becomes

$$C_2^{(l)}(3) = \frac{1}{2 \cdot 3^{2/3}} \int_1^3 [1 - (-1)^l \cos^l(2\theta_2)] \frac{(3 - p^2)}{(p^2 - 1)^{5/3}} d(p^2) \tag{79}$$

Figures 7 and 8 show the integrands for $l = 1$ and $l = 2$, respectively, as functions of p^2.

Here again, the integrations were carried out in several parts, with the method chosen to fit the behavior of the integrand in the various ranges of p^2. The four ranges are (1) $1 \leq p^2 \leq 1.025$, (2) $1.025 \leq p^2 \leq 1.050$, (3) $1.050 \leq p^2 \leq 2.90$, and (4) $2.90 \leq p^2 \leq 3.00$. In the first region, one can express $\cos \chi$ in a power-series expansion,

$$1 - \cos \chi = \left(\frac{\pi^2}{2}\right)(p - 1)^2 + 239.5\,(p - 1)^3 + \ldots, \tag{80}$$

where the constant 239.5 was obtained by requiring that, when $p^2 = 1.025$, equation (80) gives the same angle of deflection as is obtained by the use of the tables of elliptic integrals. Making use of equation (80), the integrals over the first range were evaluated analytically to give 0.0015 and 0.0029 as the contributions to $C_2^{(1)}(3)$ and $C_2^{(2)}(3)$, respectively. In order to evaluate the contributions from regions 2

and 3, the integrand of equation (79) was evaluated for p^2 equal to 1.025, 1.050, 1.075, . . . , 1.200, 1.250, 1. 300, 1.4, 1.5, . . . , 2.9. First, a four-point Lagrangian integration formula was used which corresponded to assuming that the integrand is the cubic polynomial in p^2 which fits the calculated values at $p^2 = 1.025$, 1.050, 1.075, and 1.100 and integrating this cubic from $p^2 = 1.025$ to $p^2 = 1.050$. This gave 0.0026 and 0.0052 as the contributions from the second range to $C_2^{(1)}(3)$ and

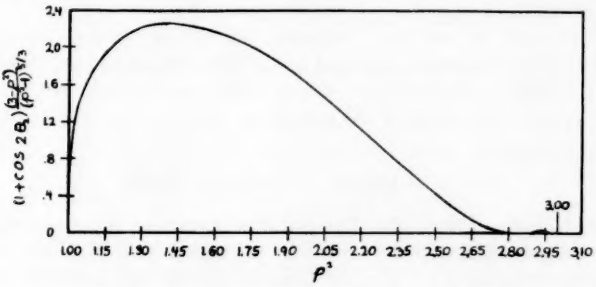

FIG. 7.—Integrand of $C_2^{(1)}(3)$ from equation (79).

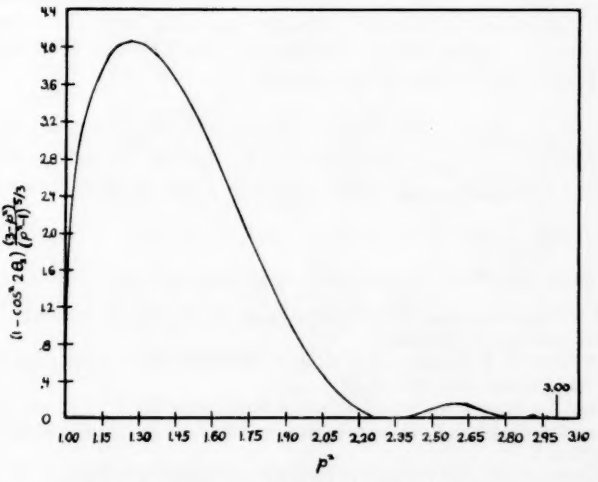

FIG. 8.—Integrand of $C_2^{(2)}(3)$ from equation (79).

$C_2^{(2)}(3)$, respectively. The contributions to these two integrals from region 3 were found by Simpson's rule to be 0.1996 and 0.2267. In region 4, cos $(2\theta_2)$ oscillates rapidly, but the maximum value of the integrand is so small that it is easy to make a sufficiently accurate approximation to the contributions, $J_4^{(1)}$ and $J_4^{(2)}$, to the two collision integrals. The largest possible value that $J_4^{(1)}$ might have is obtained by setting cos $(2\theta_2) = 1$ in the integrand of equation (79). Thus

$$J_4^{(1)} < J_{\max}^{(1)} = 3^{-5/3} \int_{2.9}^{3} (3 - p^2)\,(p^2 - 1)^{-5/3}\, d(p^2) = 0.00026. \qquad (81)$$

Since the cosine term of equation (79) has infinitely many oscillations in this region, to a sufficient degree of accuracy, the factor $(1 + \cos 2\theta_2)$ can be replaced by its mean value of unity in the integral for $J_4^{(1)}$. Thus a good estimate of $J_4^{(1)}$ is $\frac{1}{2} J_{\max}^{(1)}$, and the contribution of region 4 to $C_2^{(1)}(3)$ is taken to be 0.00013.

The best estimate of $J_4^{(2)}$ is obtained in a similar fashion. Here the maximum value of $(1 - \cos^2 2\theta_2)$ is unity, so that the maximum value of $J_4^{(2)} = \frac{1}{2} J_{\max}^{(1)}$. The average value $(1 - \cos^2 2\theta_2)$ is one-half, so that our best estimate of $J_4^{(2)}$ is $\frac{1}{4} J_{\max}^{(1)}$, or the contribution of region 4 to $C_2^{(2)}(3)$ is taken to be 0.00007.

Thus, all together, we find that $C_2^{(1)}(3) = 0.2038$ and $C_2^{(2)}(3) = 0.2349$. Combining these values with those of the previously obtained $C_1^{(1)}(3)$ and $C_1^{(2)}(3)$, we finally obtain the results

$$A^{(1)}(3) = 0.6750, \qquad A^{(2)}(3) = 0.4641.$$

c) $\delta = 6$; Attractive Potential.—The collision integrals for an attractive point-force power-law potential with $\delta = 6$ can be estimated from Mason's[4] calculations of the collision integrals, $Z^{(l,s)}$, for the Buckingham (6-exp) potential in the limit that the temperature approaches zero. In this low-temperature limit, the exponential repulsive part of the Buckingham potential does not affect the values of the collision integrals. Thus we can consider the Buckingham potential to be equivalent to the inverse sixth-power attractive potential, $\Phi(r) = -a/r^6$ with $a = r_m^6\, \epsilon/(1 - (6/\alpha))$. Interpreted in this fashion, the $Z^{(l,s)}$ for $T = 0$ can be expressed in terms of the $A^{(l)}(6)$ by the relation

$$Z^{(l,s)} = \frac{4(6)^{1/3}(1 + l)\Gamma(s + (5/3))A^{(l)}(6)}{(s + 1)!(1 + 2l - (-1)^l)}. \qquad (82)$$

Thus, from the tabulated values of $Z^{(l,s)}$ for $T = 0$, we obtain

$$A^{(1)}(6) = 0.434, \qquad A^{(2)}(6) = 0.328, \qquad A^{(3)}(6) = 0.502, \qquad A^{(4)}(6) = 0.420.$$

Mason estimates that these integrals may be in error by 1 per cent.

* This work is being done under Office of Naval Research Contract N7-onr-28511.

† Fellow, National Science Foundation.

[1] J. O. Hirschfelder, C. F. Curtiss, and R. B. Bird, *Molecular Theory of Gases and Liquids* (New York: John Wiley & Sons, Inc., 1954), p. 547.

[2] We find that for either attractive or repulsive point forces the $A^{(l)}$ (1) are infinite. The second virial coefficients (*ibid.*, p. 157) are infinite for either attractive or repulsive potentials if δ is not greater than 3.

[3] H. M. Mott-Smith, *A New Approach in the Kinetic Theory of Gases* (M.I.T. Lincoln Laboratory Group Report V-2, December, 1954). In this report Mott-Smith obtained the following values for the collision integrals for repulsive point force power potentials:

$$A^{(1)}(4) = 0.29837, \qquad A^{(2)}(4) = 0.3082, \qquad A^{(4)}(4) = 0.4216,$$
$$A^{(6)}(4) = 0.4960, \qquad A^{(1)}(12) = 0.3449.$$

[4] E. A. Mason, *J. Chem. Phys.*, **22**, 169, 1954; see also Hirschfelder *et al.*, *op. cit.*, p. 1164.

[5] Hirschfelder *et al.*, *op. cit.*, p. 546. It should be noted that our $A^{(l)}(\delta)$ and theirs are equal to $2^{-2/\delta} A_l(\delta + 1)$, where the $A_l(\delta + 1)$ are the collision integrals of S. Chapman and T. Cowling, *The Mathematical Theory of Non-uniform Gases* (Cambridge: At the University Press, 1939), p. 172.

[6] $\Omega^{(l,s)}$ as given in eq. (1) is obtained from $Q^{(l)}$ by use of the equation

$$\Omega^{(l,s)} = \left(\frac{kT}{2\pi\mu}\right)^{1/2} \int_0^\infty e^{-\gamma^2} \gamma^{2s+3} Q^{(l)} \, d\gamma,$$

where $\gamma^2 = \mu g^2/2kT$ (Hirschfelder *et al.*, *op. cit.*, p. 525).

[7] The evaluation of $A^{(1)}(2)$ and $A^{(2)}(2)$ was carried out by John S. Dahler with the help of Donald W. Jepsen.

[8] *Tables of Sine, Cosine, and Exponential Integrals*, Vol. 2 (New York: Federal Works Agency, Works Progress Administration for the City of New York, 1940).

[9] E. T. Whittaker and G. N. Watson, *A Course of Modern Analysis* (4th ed.; Cambridge: At the University Press, 1952), p. 128.

[10] D. Bierens de Haan, *Nouvelles tables d'intégrales définies* (New York: G. E. Stechert & Co., 1939), Table 161, 3.

$$\int_0^\infty \frac{\sin px}{q^2 - p^2} \, dx = \frac{1}{q} \{Ci(pq) \sin pq - Si(pq) \cos pq\},$$

with

$$Ci(pq) = \int_\infty^{pq} \frac{\cos x}{x} \, dx.$$

[11] M. A. Eliason, D. E. Stogryn, and J. O. Hirschfelder, University of Wisconsin Naval Research Report WIS-ONR-19, March, 1956. This report contains considerable calculational details not given in the present paper and includes a table of χ versus β^2 for the inverse-cube attractive potential.

[12] This change of variables was suggested to us by Donald W. Jepsen.

[13] P. F. Byrd and M. D. Friedman, *Handbook of Elliptic Integrals for Engineers and Physicists* (Berlin: J. Springer, 1954), p. 88, eq. (241.00).

[14] *Ibid.*, p. 324.

[15] *Ibid.*, p. 12, eq. (113.02).

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[17] *Ibid.*, p. 74, eq. (234.00).

ON THE APPROACH TO THE FINAL PERIOD OF DECAY IN ISOTROPIC TURBULENCE ACCORDING TO HEISENBERG'S TRANSFER THEORY

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Introduction.—The final period of decay is usually defined[1] as that period in the decay of the turbulence for which the nonlinear terms in the Navier-Stokes equation are negligible. Although this formulation of the problem does give useful information about the asymptotic form of the energy spectrum and the corresponding correlation functions, it cannot, by its very nature, deal with the *approach* to this asymptotic state during which the transfer of energy is not quite negligible. Furthermore, at the onset of the final period, the Reynolds number of the turbulence, though small, is not negligible, being of the order of 5 or so,[2] and this suggests

that a transfer of energy may exist which is compatible with the observed final-period decay law,

$$\overline{u^2} \propto (t - t_0)^{-5/2}. \tag{1}$$

Thus, if one regards the final period of decay as being *defined* by the decay law (1), rather than by the absence of energy transfer, then one can obtain not only the known properties of the energy spectrum in the final period but also, for a given transfer theory, the asymptotic form of the transfer function.

For the decay law (1), the dissipation length parameter is given by

$$\lambda^2 = 4\nu(t - t_0), \tag{2}$$

so that the Reynolds number of the turbulence, $R_\lambda = (\overline{u^2})^{1/2}\lambda/\nu$, is not constant in the final period as it is in the initial period but decreases according to the law

$$R_\lambda \propto (t - t_0)^{-3/4}. \tag{3}$$

Final Period Similarity.—Consider now the usual (exact) dynamical equation for the rate of change with time of the energy spectrum function $E(k)$ in isotropic turbulence,

$$\frac{\partial E(k)}{\partial t} = T(k) - 2\nu k^2 E(k), \tag{4}$$

where $T(k)$ is the transfer function usually denoted by this symbol. For purposes of the present discussion we shall use Heisenberg's physical transfer theory[3] to supplement equation (4); this transfer theory leads to an expression for $T(k)$ in terms of $E(k)$ which can be written in the form

$$T(k) = -2\kappa \frac{d}{dk} \int_k^\infty \sqrt{\frac{E(k')}{k'^3}}\, dk' \int_0^k k''^2 E(k'')\, dk'', \tag{5}$$

where κ is a constant of order unity.

We now assume a similarity solution of the form

$$\begin{aligned} E(k, t) &= \nu^{5/2}(t - t_0)^{-1/2} R_\lambda^2 F(x, R_\lambda), \\ T(k, t) &= \nu^{5/2}(t - t_0)^{-3/2} R_\lambda^2 U(x, R_\lambda), \end{aligned} \tag{6}$$

and

where

$$x = (\nu k^2 t)^{1/2}. \tag{7}$$

This type of similarity remains valid whatever may be the dependence of R_λ on the time and thus includes not only initial period similarity (R_λ = constant), which I have discussed elsewhere[4] along similar lines for small values of the Reynolds number, but also final period similarity with which we are concerned here.

Thus, by substituting equations (6) in equations (4) and (5), and by using the fact that $R_\lambda \propto (t - t_0)^{-3/4}$, we obtain

$$\left(x \frac{\partial}{\partial x} - \frac{3}{2} R_\lambda \frac{\partial}{\partial R_\lambda}\right) F(x) + 4(x^2 - 1) F(x) = 2U(x) \tag{8}$$

and

$$U(x) = -2\kappa R_\lambda \frac{d}{dx}\int_x^\infty \sqrt{\frac{F(x')}{x'^3}}\,dx' \int_0^x x''^2\,F(x'')\,dx''. \tag{9}$$

The Asymptotic Solutions.—For small values of the Reynolds number, equation (9) shows that $U(x)$ is of smaller order than $F(x)$. Accordingly, we can assume a solution in the form of a power series in the Reynolds number of the form

$$\text{and} \qquad \begin{aligned} F(x, R_\lambda) &= \sum_{n=0}^{\infty} F_n(x)(\kappa R_\lambda)^n, \\ U(x, R_\lambda) &= \sum_{n=1}^{\infty} U_n(x)(\kappa R_\lambda)^n. \end{aligned} \tag{10}$$

Since $R_\lambda \propto (t - t_0)^{-3/4}$, this power series expansion in the Reynolds number is equivalent to an asymptotic expansion in the time, and it is this fact which makes a solution of the form (10) meaningful in the final period of decay; for, with an increasing Reynolds number, we are simply moving backward in time.

By substituting the series (10) into equations (8) and (9) and by equating like powers of R_λ, one is led to a system of equations from which the functions $F_n(x)$ and $U_n(x)$ can be obtained by quadrature. From the relation

$$\overline{u^2} = 2/3 \int_0^\infty E(k)\,dk, \tag{11}$$

the boundary conditions on the functions $F_n(x)$ follow in the form

$$\int_0^\infty F_n(x)\,dx = \begin{cases} 3/8 & (n = 0), \\ 0 & (n \geq 1), \end{cases} \tag{12}$$

which show that the general shape of $F(x)$ is determined by $F_0(x)$ and that the functions $F_n(x)$, for $n \geq 1$, cause only a detailed modification of that shape as the Reynolds number is increased and leave the decay law (1) unaffected.

The functions $F_0(x)$ and $U_1(x)$ evaluated in this way are given by

$$F_0(x) = 4(2/\pi)^{1/2}\,x^4 e^{-2x^2} \tag{13}$$

and

$$U_1(x) = -16\left(\frac{2}{\pi}\right)^{3/4}\frac{d}{dx}\int_x^\infty x'^{1/2}\,e^{-x'^2}\,dx' \int_0^x x''^6\,e^{-2x''^2}\,dx'', \tag{14}$$

which are shown graphically in Figure 1.

The Skewness Factor.—From the foregoing solution it is now possible to calculate the skewness factor of $-\partial u_1/\partial x_1$,

$$S = -\frac{\overline{(\partial u_1/\partial x_1)^3}}{\left[\overline{(\partial u_1/\partial x_1)^2}\right]^{3/2}}. \tag{15}$$

What is perhaps most surprising about this final-period solution is that S does not tend to zero asymptotically in the final period as one might have expected but ap-

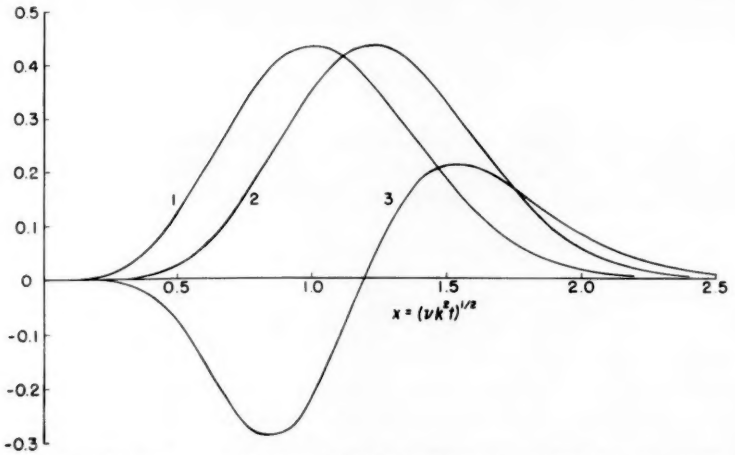

FIG. 1.—The distribution of energy transfer in the final period of decay according to Heisenberg's transfer theory. Curve *1*, the energy spectrum, $F_0(x)$; curve *2*, the energy-dissipation spectrum, $0.8054x^2 F_0(x)$; curve *3*, the transfer function, $2U_1(x)$.

proaches a nonzero constant value. This can easily be seen by writing equation (15) in the alternative form

$$S = \frac{3}{14}(30)^{1/2}\frac{\int_0^\infty k^2 T(k)\,dk}{[\int_0^\infty k^2 E(k)\,dk]^{3/2}}. \tag{16}$$

In terms of the similarity functions, equation (16) becomes

$$S = \frac{3}{14}(30)^{1/2}\frac{1}{R_\lambda}\frac{\int_0^\infty x^2 U(x)\,dx}{[\int_0^\infty x^2 F(x)\,dx]^{3/2}}, \tag{17}$$

which shows that, as $R_\lambda \to 0$,

$$S/\kappa = 128/35\int_0^\infty x^2 U_1(x)\,dx + O(\kappa R_\lambda). \tag{18}$$

A numerical evaluation of the integral occurring in this equation then shows that, as $R_\lambda \to 0$,

$$\frac{S}{\kappa} \to 0.5764. \tag{19}$$

Thus, provided that κ is not Reynolds number dependent—a possibility that can hardly be admitted—the skewness factor approaches a constant value in the final period.

It is of some interest to compare the value of S/κ obtained here in the final period of decay with the initial period values[4] of 0.78 ($R_\lambda \to 0$) and 1.52 ($R_\lambda \to \infty$). These results suggest, then, that the value of S/κ varies by less than a factor of 3 throughout the entire life history of the turbulence, whatever may be the value of the Reynolds number.

Finally, it should be remarked that in the final period the individual Fourier coefficients of the velocity field, though statistically dependent, do not interact

but simply decay through direct viscous action.[5] The present calculation then shows explicitly that this statistical dependence between the different Fourier coefficients does exist and persists but that, for Heisenberg's theory, it has a universal character determined only by the transfer mechanism.

[1] G. K. Batchelor, *The Theory of Homogeneous Turbulence* (Cambridge: At the University Press, 1954).

[2] G. K. Batchelor and A. A. Townsend, "Decay of Turbulence in the Final Period of Decay," *Proc. Roy. Soc. London, A*, **194,** 527–543, 1948.

[3] W. Heisenberg, "Zur statistischen Theorie der Turbulenz," *Z. Physik*, **124,** 628–657, 1948.

[4] W. H. Reid, "Two Remarks on Heisenberg's Theory of Isotropic Turbulence," *Quart. Appl. Math.* **14,** 201–205, 1956.

[5] Cf. M. J. Lighthill, *Nature*, **173,** 746, 1954. I am indebted to Professor Lighthill for some further illuminating remarks regarding this point.

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*ON THE CLASSIFICATION OF REINFORCEMENT SCHEDULES**

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Schedules of reinforcement for operant behavior have ordinarily been classified under two major headings: those based on the passage of specified time intervals of nonreinforcement between reinforced responses and those based on the emission of specified numbers of responses by the organism. Skinner describes this distinction as one between schedules which "are arranged by a system outside the organism [fixed and variable interval schedules] and those which are controlled by the behavior itself [fixed and variable ratio schedules]."[1] Both types of schedule have in common a periodicity or intermittence of reinforcement, but some of the differences in their behavioral effects are striking.[2] In view of the differential effects observed on behavior and the different procedures followed by the experimenter, the two categories are regarded as distinct by most, if not all, theorists. Nevertheless, it would seem worth while if such an integration could be made, and an attempt in this direction is offered in the present paper.

We may consider that a schedule of reinforcement acts to maintain behavior through the differential reinforcement of a particular pattern of responses in time (or, equivalently, single responses having certain temporal characteristics with respect to other responses). From this viewpoint the "count" involved in a "ratio" schedule may be only incidental to the generation of a maximal rate of responding, with the crucial factor being the increased probability of reinforcement for responses following each other at short intervals. By implication, then, if external conditions were arranged to favor rapid responding by the organism, we might expect to observe the "bursts" (short periods of high response rate) and "breaks" (pauses in responding) so characteristic of "ratio" reinforcement. "Interval" schedules, on the other hand, may differentially reinforce responses preceded by relatively longer intervals of no responding, producing the response rates and temporal distributions characteristic of these schedules. Skinner has

noted that "schedules are simply rather inaccurate ways of reinforcing rates of responding."[3] If these schedules are so regarded, there is no reason to believe that their integration into one conceptual framework is impossible.

The projected classification of reinforcement schedules utilizes "time-sampling" contingencies, in the description of which we shall employ the terms t^D and t^Δ. The term t^D represents a period of time during which a specified instrumental response of the organism may be followed by a reinforcing event; t^Δ represents a period of time during which this response will not be followed by a reinforcing event. A fundamental case may be established with these three restrictions: (1) t^D and t^Δ are held constant, (2) t^D and t^Δ are alternated, and (3) only the first response in t^D is reinforced. It may be noted that reinforcements can easily be "missed" on such a schedule; if the organism does not respond at all during the t^D period, it does not receive a reinforcement during that $t^D + t^\Delta$ cycle.

The experimental domain suggested by these variables may be visualized as a co-ordinate system with the duration of t^D along one side and the duration of t^Δ along the other. Increasing either t^D or t^Δ alone changes both the total cycle length, $t^D + t^\Delta$, and the proportion of the cycle during which a response may be reinforced, $t^D/(t^D + t^\Delta)$. The experimental domain may then be mapped as in Figure 1, wherein are schematized certain sectors which may represent different behavioral effects correlated with classically defined "interval" and "ratio" schedules. It should be remembered that the areas and boundaries of the sectors are merely suggestive; their existence and limits (probably not sharp) remain to be empirically determined in terms of the behavioral properties exhibited.

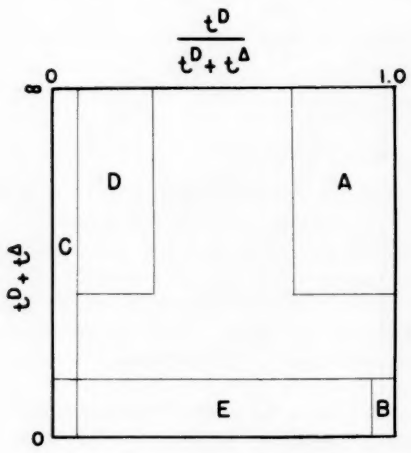

FIG. 1.—For explanation see text.

Fixed Interval Schedules.—This case is marked by $t^D/(t^D + t^\Delta)$ approximating or equaling unity (where $t^\Delta \to 0$ and $t^D > 0$) and comparatively long cycle lengths. If $t^D/(t^D + t^\Delta) = 1$, the organism can "miss" a reinforcement only in the sense that a whole cycle may pass without a response, with one reinforcement opportunity lost, though the next subsequent response is reinforced whenever it occurs. When $0 < t^D/(t^D + t^\Delta) < 1$, "missing" of a reinforcement can take place in the same way, but it can also occur when the first response in a cycle is made in t^Δ. With $t^D + t^\Delta$ long enough, these schedules permit the development of a temporal discrimination, as shown by a varying "density" of response in which the organism's interresponse intervals do not remain random but systematically decrease in any cycle. These schedules are schematically located in sector *A* of Figure 1.

Regular Reinforcement and Extinction.—"Regular reinforcement" is obtained with $t^\Delta = 0$ and with cycle lengths (composed entirely of t^D) shorter than the duration of a reinforcement. Thus, if a pigeon is allowed 3 seconds' access to

grain as a reinforcement, a t^D equal to 2 seconds and $t^\Delta = 0$ will insure that a new cycle has begun by the time the bird has finished eating, and every response will be reinforced (see sector *B*, Fig. 1).

"Extinction" is the case where $t^D \to 0$ and $t^\Delta > 0$ ($t^D/(t^D + t^\Delta) \to 0$). The subject is reinforced so infrequently that behavior cannot be maintained (see sector *C*, Fig. 1).

Ratio Schedules.—The incorporation of response contingency ("ratio") schedules into the proposed framework apparently presents greater problems than the above, since the co-ordinates of Figure 1 are temporal in nature. Several considerations, however, help to resolve the apparent incompatibility between the two metrics of time and response number. By reducing $t^D/(t^D + t^\Delta)$ to a sufficiently small value and keeping reasonably long cycle lengths, we favor the adoption by the organism of a high response rate, since on such a schedule reinforcement is more probable or frequent following short interresponse intervals (i.e., high rates). Sector *D* of Figure 1, by such reasoning, would include t^D and t^Δ values which might be expected to yield behavioral effects typical of fixed ratio and some variable ratio schedules. It is an empirical question whether choices of such t^D and t^Δ values would result in "ratio" behavior ("breaks" and "bursts," etc.) and whether sudden large decreases in $t^D/(t^D + t^\Delta)$ would reproduce the known effects[2] of switching an organism to a much higher fixed or mean variable ratio. Some preliminary data will be presented below indicating that such is, indeed, the outcome.

Certain variable ratio schedules, which might be termed "random ratio," specify the probability of reinforcement for any particular response; a 0.25 random ratio, for example, indicates that there is a probability of 0.25 that any single response will be reinforced. The rates of responding generated by such schedules are particularly stable and are free of the "breaks" after reinforcement.[4] In the present framework, as cycle lengths become shorter, such rapid alternations of t^D and t^Δ are eventually reached that "breaks" after reinforcements should disappear, because responses immediately after reinforcement are as likely to be reinforced as are any other responses. In this case, the probability of reinforcement for any response, as with "random ratio" schedules, can be specified by the proportion $t^D/(t^D + t^\Delta)$. For example, with short cycle lengths, perhaps of the order of less than a second, and with $t^D/(t^D + t^\Delta)$ equal to $1/20$, there is on the average one chance in twenty of a given response's being reinforced. Sector *B*, "regular reinforcement," fits into sector *E*, "random ratio reinforcement," and accounts for cases where the probability of reinforcement is unity for each response. Sector *C*, "extinction," is also included in sector *E* and covers the case where there is a probability of zero that any response be reinforced. Some preliminary results displaying "random ratio" response features, for schedules assignable to sector *E*, will be presented below.

It may be noted that "breaks" after reinforcement are observed in high "fixed ratio" schedules and "variable ratio" schedules with a high mean ratio but become negligible during random and low mean variable ratio schedules of reinforcement. We would expect a similar difference to exist between sectors *D* and *E* of Figure 1; in sector *D* (presumably fixed and high mean variable ratio schedules) the cycle length is long enough so that responses immediately after reinforcements are almost never reinforced, while in sector *E*, as remarked earlier, the short cycle

length makes "breaks" after reinforcement unlikely. Additionally, we might expect that once the cycle length goes below a critical small value, cycle length should cease to be an important variable; the organism's responding is then affected only by the $t^D/(t^D + t^\Delta)$ value prevailing.

The areas of Figure 1 as yet unmentioned cannot now be categorized in any systematic way with regard to their behavioral effects, and they may represent schedules of reinforcement not falling within conventional categories. Empirical determinations in these areas may indicate a blending of "interval" and "ratio" behavior.

Other Schedules.—The three restrictions underlying Figure 1 are not the only possibilities. Each represents a special case of one or more variables which might themselves be systematically manipulated. For example, a schedule may be constructed in which t^D and t^Δ are not held constant but are varied according to some program consisting, perhaps, of a prearranged contingency table governing the probability at any time of passing from one part of the domain to another. If such a program were designed incorporating a high probability of arriving at and staying in the "interval" area of Figure 1 (sector A) and a low probability of staying within any single portion of that area, we should expect the resultant behavior to approximate that appropriate to "variable interval" schedules. In like manner, a program which kept the schedule of reinforcement within sector D of Figure 1 should yield behavior appropriate to a "variable ratio" schedule though possibly not to "random ratio." Greater complexity in behavioral properties might result from a program not limited to a particular sector of Figure 1.

We may wish, in addition, to alter the other two restrictions underlying Figure 1. For example, it would be possible to present randomly, rather than by regular alternations, a fixed t^D and a fixed t^Δ. Further, it would be possible to specify any desired probability of reinforcement of other than the first, or more than one, response in t^D. In fact, the entire analysis up to now could be duplicated in any t^D period by including within t^D itself any "interval" or "ratio" schedule so far discussed. Obviously, too, all the usual independent variables of behavioral research, such as drive, could figure as parameters of the present classification.

SOME EXPERIMENTAL RESULTS

With the end in mind of experimentally exploring the above schema, an apparatus was designed and constructed, yielding a range of $t^D/(t^D + t^\Delta)$ values from less than 0.005 to 1.00, with cycle lengths variable from $^1/_8$ second to approximately 4 minutes. The subjects for these experiments were female white Carneaux pigeons about six years of age. In order to insure a constant "drive level," the birds were maintained throughout the experiment at 80 per cent (± 15 gm.) of their weight under ad libitum feeding. The pigeons were trained to peck at a key, or trans-illuminated disk, at one end of the experimental cage; during preliminary training, each key-pecking response was reinforced by the presentation for 3 seconds of a small grain hopper several inches beneath the key. For a more detailed description of the experimental cage and conditioning technique see the account of these procedures given by Ferster.[5] Response data were taken in the form of cumulative response curves, total number of responses and reinforcements per session, and polygraph records of response distributions in time.

Two types of studies from which the present data are taken are progressing in this laboratory. These data will be presented in greater detail in subsequent publications. In one, $t^D/(t^D + t^\Delta)$ is maintained at a constant value while cycle length is systematically varied. In the second, part of a doctoral dissertation by one of the authors, the length of t^D is varied while total cycle length is maintained constant. On each schedule studied, the original three restrictions apply: t^D and t^Δ are held constant, t^D and t^Δ are alternated, and only the first response in t^D is reinforced. After initial training of the key-pecking response, all birds were placed on a schedule permitting the first response in any 30-second period to be reinforced ($t^D/(t^D + t^\Delta) = 1.00$, $t^D + t^\Delta = 30$ seconds), a schedule identical with a 30-second "fixed interval."

After approximately fourteen days of preliminary training on the above intermittent schedule, birds in the first experiment were shifted to a new schedule, in which the total cycle length was 30 seconds and t^D was 1.5 seconds ($t^D/(t^D + t^\Delta) = 0.05$). $t^D/(t^D + t^\Delta)$ was held constant at 0.05 throughout this study. Data are being taken on cycle lengths of 30, 15, 7.5, 3.75, 1.88, and 0.94 seconds. Each bird is kept on a given schedule until its response rate has reached a steady state, as defined by the following stability criterion. The first seven days on any schedule are not considered in computing stability. For the next six days the mean of the first three days of the six is compared with that of the last three days; if the difference between these means is less than 5 per cent of the six days' mean, the bird is considered to have stabilized and is shifted to the next schedule. If the difference between submeans is greater than 5 per cent of the grand mean, another experimental day is added and similar calculations are made for that day and the five immediately preceding it. Such extensions of the experiment and calculations of stability are continued daily until the bird reaches the afore-mentioned 5 per cent criterion. Though each bird spends 20 minutes daily, weight permitting, in the experimental box, only the last 15 minutes are employed in calculating stability criteria.

Figure 2 displays typical cumulative response curves of bird No. 8 for complete 20-minute sessions late in training on each schedule with cycle lengths of 30, 15, 7.5, and 3.75 seconds and respective t^D's of 1.5, 0.75, 0.38, and 0.19 seconds. Curves are not shown for cycle lengths of 1.88 and 0.94 seconds, since these, as it turned out, would be superimposed on the 3.75-second curve. Four birds are being used in this study, and all have exhibited consistent functions. With long cycle lengths the rate of responding is relatively low; as the cycle length decreases, rate of response increases, appearing to approach an asymptote at about 3.75 seconds. "Fixed interval" responding is displayed when the total cycle length is 30 seconds. As the cycle is shortened to approximately 2 seconds, however, the bird's response output develops features characteristic of "random ratio" behavior and approaches a mean value of 20 responses per reinforcement.

The second current investigation involves an examination of the effects of decreasing $t^D/(t^D + t^\Delta)$ from 1.00 through 0.35, 0.05, 0.03, 0.02, and 0.013, while maintaining cycle length constant at 30 seconds. Each bird is used daily, weight permitting, for a total of 30 minutes. Figure 3 indicates representative cumulative response curves of bird No. 3 on the enumerated $t^D/(t^D + t^\Delta)$ values. When $t^D/(t^D + t^\Delta)$ equals 1.00, the cumulative curve is marked by temporal discrimina-

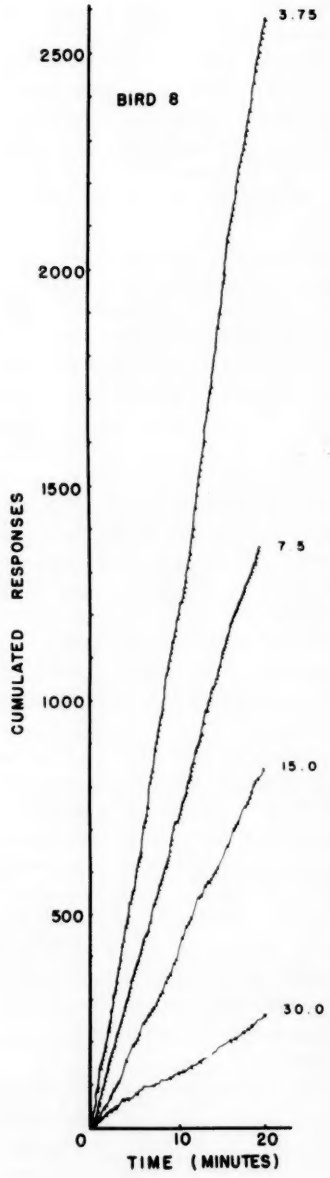

FIG. 2.—Typical cumulative response curves for bird No. 8. The value of $t^D + t^\Delta$ is shown at the end of each curve. In all cases $t^D/(t^D + t^\Delta) = 0.05$. Reinforcements are indicated by short diagonal lines.

tions typical of responding under "fixed interval" schedules. As the t^D period shortens, the response rates increase and the general shape of the curves becomes more similar to that under "fixed ratio" or high mean "variable ratio" schedules.

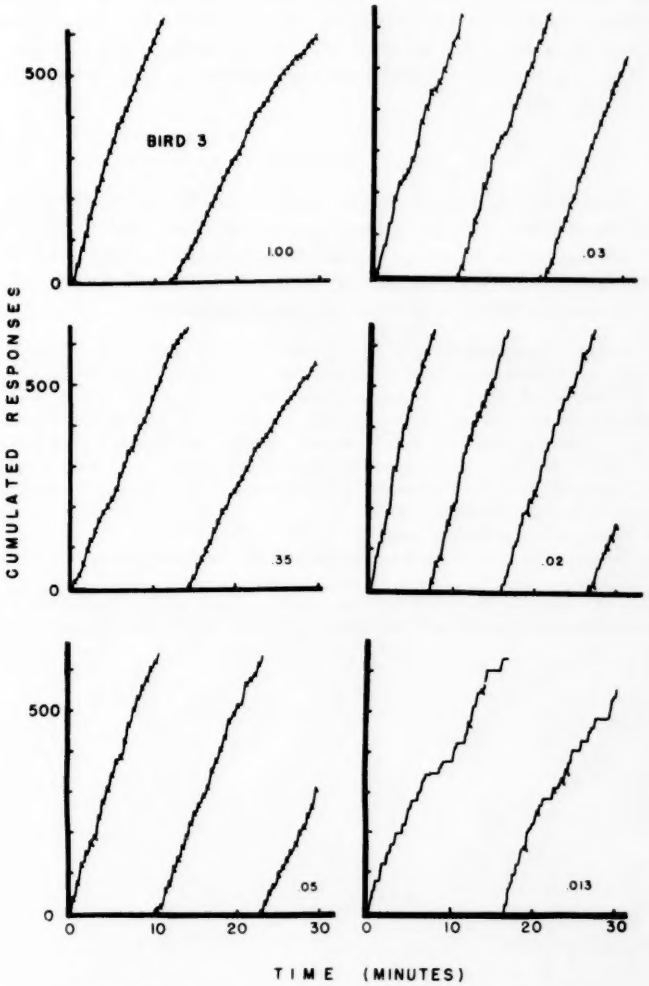

FIG. 3.—Typical cumulative response curves for bird No. 3. In order to conserve space, the curves are displaced to the base line after approximately 650 responses have accumulated. The value of $t^D/(t^D + t^\Delta)$ is shown for each curve. In all cases $t^D + t^\Delta = 30$ seconds. Reinforcements are indicated by short diagonal lines.

"Breaks" after reinforcements appear to increase with a decrease in t^D, a finding already known to be the outcome of successive increases in the magnitude of the fixed ratio.[6] The last curve ($t^D/(t^D + t^\Delta) = 0.013$) resembles that obtained by

Skinner[2] after shifting subjects from one "fixed ratio" to a much higher "fixed ratio." Three other birds employed in this second study show similar changes from "fixed interval"-like response curves to "ratio"-like curves and an increase in response rate as $t^D/(t^D + t^\Delta)$ decreases. With continued shortening of t^D, perhaps beyond values used in this study, extinction of the response would eventually occur, since the probability of coincidence between response and t^D would fall so low (at any actually attainable rate of response) as to make the maintenance of behavior impossible.

SUMMARY

An attempt was made to show how operant reinforcement schedules may be viewed as special cases of one general conceptual framework employing certain temporal parameters as its major independent variables. Some preliminary data were presented to show how shifts from "interval" to "ratio" response characteristics can be obtained by varying these temporal parameters.

* This research was supported in part by a grant from the Eugene Higgins Fund.

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[2] B. F. Skinner, *The Behavior of Organisms* (New York: D. Appleton–Century Co., 1938).

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[4] C. M. Brandauer, unpublished data.

[5] C. B. Ferster, "The Use of the Free Operant in the Analysis of Behavior," *Psychol. Bull.*, **50**, 263–274, 1953.

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